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(54) Title: HAPTENATED PEPTIDES AND USES THEREOF

(57) Abstract

MHC-binding carrier peptides linked to catechol derivatives are provided which are recognized by urushiol-specific T lymphocyte. The compounds are useful in therapeutic compositions and methods for desensitizing individuals against contact sensitivity to haptens, such as urushiol of poison ivy/poison oak.

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HAPTENATED PEPTIDES AND USES THEREOF

5 Background

Delayed-type hypersensitivity (DTH) is an inflammatory immune reaction mediated by T lymphocytes. DTH reactions were first observed in persons inoculated for a second time with cowpox, who developed a reddish, painful swelling on the skin at the site of inoculation. Similar observations were made by Robert Koch in 1890 in
10 persons with tuberculosis: Whereas normal persons showed no reaction after subcutaneous injection of tuberculin, those previously exposed to tuberculin developed a red, weeping skin lesion at the injection site, which reaction increased for 24 to 72 hours, then disappeared. This tuberculin DTH reaction became the standard diagnostic test for tuberculosis.

15 A DTH reaction can occur only in an individual previously exposed (sensitized) to a given antigen. The manifestation of the reaction can be localized (the skin reaction at the site of injection) or systemic (whole body). Localized DTH reactions can be elicited by subcutaneous injection of an antigen to which the individual has been previously exposed. Within 6 to 8 hours, the skin at the site of
20 the injection becomes red, warm and swollen. The swelling and characteristic hardness of the flesh are due to a massive infiltration of monocytic cells, particularly macrophages, and lymphocytes. Lysosomal hydrolases, lymphotoxin, and other enzymes and factors released by the infiltrating cells damage surrounding tissues and account for the redness and necrosis of the tissue surrounding the injection site.
25 Systemic DTH reactions occur when large amounts of the antigen are introduced into the blood stream. Typical symptoms include fever, painful joints, and lymphopenia, but severe cases may result in shock or even death several hours after antigen injection.

 Contact sensitivity (contact dermatitis) is a form of delayed-type
30 hypersensitivity caused by contact of a sensitizing substance with the skin, to which the skin develops an inflammatory reaction. The substances capable of inducing contact sensitivity typically are compounds of low molecular weight, which permits them to diffuse through the skin, and are compounds which are not antigenic by themselves but form conjugates with proteins inside the body, which conjugates, in
35 turn, are antigenic, i.e., capable of stimulating lymphocytes. Such compounds are termed *haptens*.

Haptens responsible for contact sensitivities have been detected in a wide range of industrial and natural products, including mercuric compounds in ointments, chromate, nickel, turpentine, varnishes, resins, cosmetics and dyes. Experimentally, contact sensitivity has been induced using substituted benzenes, e.g., picryl chloride, 3,4-dinitrochloro-benzene, azobenzenearsonate, dinitrophenol (DNP), trinitrophenol (TNP), etc. (See, generally, Klein *Immunology: The Science of Self and Non-self Discrimination*, 1982 (John Wiley & Sons, Inc., New York), section 10.1.6 (Haptens) pp. 357-360.)

A prime example of naturally occurring haptens is urushiol, found in poison ivy, poison oak, primrose, uncured Japanese lacquer and poison sumac. Exposure to urushiol-containing oils exuded by these plants sensitizes a susceptible individual, and re-exposure to the sensitizing agent can induce irritation, inflammation and blistering of the skin. Severe reactions can require hospitalization or can even be fatal. (See, Kligman (1958) *AMA Arch. of Dermatology* 77:149-180).

Urushiol is a family of catechol derivatives, made up of compounds having a catechol nucleus with a C₁₅ or C₁₇ hydrocarbon side chain attached at the 3 position of the catechol ring. The side chains can be saturated (e.g., 3-*n*-pentadecyl catechol and 3-*n*-heptadecyl catechol) or unsaturated. (See, ElSohly et al. (1982) *J. Nat. Prod.* 45: 532-538). Immunologically, urushiols function as haptens, in that they are not recognized by the immune system unless they are bound to a carrier molecule. Upon exposure of the epidermis to urushiol, the compound forms a covalent linkage with (or "haptenate") endogenous proteins. The haptenate proteins are most likely recognized and processed by macrophages, then presented as antigens in a complex with major histocompatibility complex (MHC) molecules, which complexes, in turn, are recognized by lymphocytes, leading to a classic DTH response.

Currently the most effective means to prevent contact sensitivities and other hapten-mediated hypersensitivities is to avoid contact with the hapten. In many cases, however, such as with common industrial products (e.g., turpentine, cosmetics) or natural haptens (e.g., urushiol), avoiding the hapten may be impossible for certain individuals, particularly if their profession involves contact with environments where haptens to which they are sensitive are prevalent. (See, e.g., with respect to poison ivy/oak, Oltman and Hensler (1986) *Clinics in Dermatology* 4:213-216.)

Many treatments have been proposed for urushiol sensitivity, but most rely on barrier methods or amelioration of symptoms. See, e.g., U.S. Patent Nos. 4,259,318 (Duhe et al.), 4,451,453 (Lay et al.), 4,663,151 (Waali), 5,011,689 (Misenko), and 5,017,361 (Beall et al.), incorporated herein by reference.

Tolerization against urushiol sensitivity has been studied by several investigators. See, e.g., Watson et al. (1981) *J. Pharm. Sci.* 70(7): 785-9; Stampf et al. (1986) *J. Invest. Dermatol.* 86(5): 535-8; Dunn et al. (1987) *J. Invest. Dermatol.* 89(3):296-8. Because urushiol itself is quite toxic, tolerization has typically been attempted with better tolerated analogs, such as 3-*n*-pentadecylcatechol acetate (see, e.g., Watson et al. (1980) *J. Invest. Dermatol.* 76(3):164-70). Such compounds have several disadvantages, however, including rapid clearance, toxicity, low potency or poor induction of hyposensitivity to the natural hapten, and short-lived tolerizing effects.

Compositions and methods for tolerizing individuals against urushiol-induced contact dermatitis have been proposed by ElSohly et al., U.S. Patent No. 4,428,965 (incorporated herein by reference) which involve the coupling of catechol derivatives to cell membrane residues (i.e., cell membrane residues from autologous red blood cells); however such compositions present difficulties in providing uniform pharmaceutical formulations, and the methods are impractical in that separation of autologous cells and coupling the catechol derivatives to the cell membranes are necessary prior steps to obtaining a useful composition. In addition, the reliance on autologous cell membranes as a carrier for the catechol compound means provision of a unique medicine for each individual treated, and no compositions for general use by the urushiol-sensitive population are provided. Also, as with any medicine incorporating a blood product, stringent sterile processing is required e.g., to eliminate components that may carry blood-borne viruses and infections, such as hepatitis B. Accordingly, there is a need for more effective, highly specific and generally administrable compositions and therapies for treating contact sensitivity to urushiol and DTH-inducing haptens in general.

Summary of the Invention

The present invention provides a family of compounds for the treatment or prevention of contact sensitivity to haptens, especially plant-derived haptens such as urushiol. The present invention also provides methods for desensitizing mammals (including humans) to contact sensitivity-inducing haptens, especially plant-derived haptens such as urushiol.

Compounds according to the invention generally have two components: (A) a carrier capable of binding to Class I or Class II major histocompatibility complex (MHC) antigens, and (B) one or more hapten molecules, covalently bound to the peptide carrier. They may be represented by the general formula:

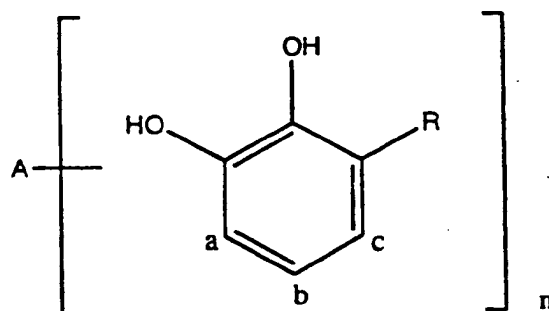
(I)



wherein A preferably represents a peptide of from 7 to 30 amino acid residues capable of binding to a Class I or Class II MHC molecule, or a peptidomimetic of similar function, B represents a hapten molecule, n represents 1, 2 or 3, and — represents a covalent bond linking each hapten molecule (B) to a functional moiety of the carrier (A) (e.g. an amino acid residue of the carrier peptide).

Preferred compounds according to the invention have the formula:

10 (II)



wherein R is a saturated or unsaturated hydrocarbon radical of from 0 to 20 carbon atoms (0 carbon atoms signifying that there is no R substituent at all), preferably 4 to 15 carbon atoms, most preferably 6 or 10 carbons atoms; A preferably represents a peptide of from 7 to 30 amino acid residues capable of binding to a class I or Class II MHC molecule; n represents 1, 2 or 3, preferably 1; and — represents a covalent bond linking each of the catechol moieties (bracketed in the formula) to an amino acid residue of the carrier peptide (A) at one of the unsubstituted positions a, b or c of the catechol ring. The R substituent may be branched, unbranched or cyclic, but is preferably unbranched.

The haptenated peptide compounds above can be administered (usually in a pharmaceutically acceptable carrier or diluent) to mammals with a sensitivity (or at risk of developing a sensitivity) to contact allergens containing haptens to desensitize them to the hapten. Particularly, the compounds of formula (II) can be used to desensitize mammals, i.e., eliminate or at least suppress (reduce the severity of) the hypersensitivity reaction, to urushiol-induced contact dermatitis.

Brief Description of the Drawings

Fig. 1 shows selected human MHC Class II binding peptides, designed in accordance with the criteria for a suitable carrier peptide in accordance with the invention.

Fig. 2 shows selected murine MHC Class II binding peptides suitable as carriers in accordance with the invention, as well as the solubility of each peptide in PBS and the affinities of each peptide for the two H-2^k class II proteins I-A^k and I-E^k

Fig. 3a-b is a graphic representation showing measurements of PDC skin
5 painted induction of DTH (as indicated by ear swelling) alone, and when pretreated with peptide :PDC conjugates: Fig. 3a depicts the response in C3H/HeN mice and Fig 3b depicts the response in B10.A(4R) mice.

Fig. 4a-d is a graphic representation showing the proliferative response of T
cell (measured in CPM) from lymph nodes (LN) of mice either treated so as to induce
10 T cell down regulation or untreated mice. 4a-b depict responses to various carriers and carrier:PDC conjugates of A5:7:PDC immunized mice (untreated), in C3H/HeN mice and B10.A(4R) mice respectively. Panel C and D present the effect of pretreatment with A5:7:PDC prior to immunization with this conjugate, on the T cell response of C3H/HeN and B10.A(4R) mice, respectively (treated).

15 Fig. 5a-c is a graphic representation of hapten specific T cell responses in primary cultures from three PBL individuals with recent exposure to poison oak. T cell proliferation is measured in counts per minute (CPM).

Fig. 6a-e is a graphic representation specific T cell response in a
desensitization experiment. Fig. 6a shows specific T cell response of A5:7:PDC
20 primed mice treated with A5:7:PDC and challenged with A5:7:PDC in the presence of adjuvant. Fig. 6b is the negative control for the experiment showing specific T cell response of A5:7:PDC primed mice, treated with PBS and challenged with A5:7:PDC in the presence of adjuvant. Fig 6c is the positive control for the experiment showing specific T cell response of A5:7:PDC primed mice treated with A5:7 peptide only and
25 challenged with A5:7:PDC in the presence of adjuvant. Fig. 6d is a tolerization experiment performed at the same time showing specific T cell response of unprimed mice tolerized with A5:7:PDC and challenged with A5:7:PDC in the presence of adjuvant. Fig. 6e shows specific T cell response of unprimed and intolerized mice challenged with A5:7:PDC in the presence of adjuvant.

30

Detailed Description of Invention

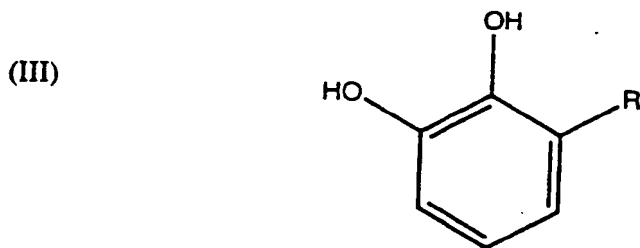
The compounds (I) of the present invention are prepared by covalently
coupling one or more hapten molecules to a carrier such as an MHC Class I or Class
II-binding peptide, to obtain a peptide/hapten conjugate capable of forming an antigen
35 complex with MHC Class I or Class II molecules, which complex, in turn, is recognized by a specific population of hapten-specific T cells. That is, the hapten/carrier compounds of the present invention, complexed with MHC Class I or

Class II molecules, bind to the T cell receptor (TCR) of T cells of an individual sensitized to the particular hapten. Such recognition ordinarily leads to T cell proliferation and the characteristic release of mediators, such as IL-2, however it is contemplated that compounds according to the invention will be administered in
5 absence of adjuvants, aggregation or other cell stimulatory signals, so as to lead to non-stimulatory recognition by the hapten-specific T cells. In this way, the compounds of the present invention can be used to disrupt the normal proliferation of hapten-specific T cells or alter the T cell-mediated DTH response to the hapten, resulting effectively in desensitization to the hapten. Whether the specific mechanism
10 of action of these compounds *in vivo* involves TCR blockade, induction of anergy, signaling of apoptosis, augmentation of T cell reactivity, induction of T cell non-responsiveness, or some other theoretical mechanism is not critical to this invention. Properly selected compounds of formula (I) can be administered to a hapten-sensitive individual to desensitize it, i.e., reduce or eliminate the DTH reaction, to the particular
15 hapten the individual sensitized to.

The compounds and methods of this invention will now be described in detail with reference to a preferred class of compounds (formula (II)) and specific embodiments. It should be recognized, however, that the principles and methods used to describe the preferred embodiments may be extended from this disclosure to a wide
20 range of hapten/carrier conjugates that will find uses in alleviating sensitivity to a variety of haptens.

Compounds of the formula (II), above, may be prepared by reacting a carrier preferably a peptide or peptidomimetic with a hapten-containing reagent to form a covalent linkage between the two. When the carrier is a peptide or appropriate
25 peptidomimetic, it is possible to react the hapten-containing reagent with the amino-terminal end of the peptide carrier, however this is not preferred, as the resulting hapten substituent may be hindered sterically from proper presentation to T cells. Care is ordinarily taken to block the terminal groups of the carrier peptide, e.g., by acetylation of the amino-terminal group and amidation of the carboxyl terminus.

30 The haptenic component of the compounds of formula (II) are preferably catechol derivatives having the general formula:



In the above formula (III), R is a C₀₋₂₀ hydrocarbon substituent which may be branched, unbranched, or cyclic, and may be saturated or unsaturated. For example, R may be *n*-alkyl or *n*-alkenyl, branched alkyl or branched alkenyl, cycloalkyl or cycloalkenyl, phenyl, naphthyl, phenylalkylene, cyclohexylalkylene, etc. Preferred substituents will be unbranched hydrocarbon side chains of 8 to 15 carbons. Particularly preferred substituents will be limited to fewer than about 10 carbon atoms, most preferably at least 8 carbon atoms, where decreasing the hydrophobicity of the side chain is desired: Natural urushiol haptens have a long unbranched side chain of 15 or 17 carbons, which is extremely hydrophobic; and it is believed that this hydrophobicity may play a role in the reactivity of the molecule, e.g., by embedding itself by this side chain in the hydrophobic lipid bilayer of cell membranes. On the other hand, where the hydrophobic nature of the substituent is less of a concern, particularly preferred compounds will employ substituents that are the same as natural urushiol, i.e., *n*-alkyl or *n*-alkenyl substituents of fifteen or seventeen carbons. Of particular interest is *n*-pentadecyl catechol (PDC), i.e., the catechol derivative of formula (III) wherein R is *n*-pentadecyl.

Catechol compounds can be synthesized using techniques known in the art. For example, PDC can be synthesized according to the procedure of Dawson and Ng, *Org. Prep. Proc. Int.* (1978) 10:167-172, or by that procedure with the modifications as described in the working examples, below. Heptadecyl catechol (HDC) can be synthesized in an analogous manner. Alternatively, urushiols can be isolated from plant tissue (see, e.g., ElSohly, M.A. et al. (1982) *J. Natural Products* 45:532-538). The catechol compounds are then reacted with a peptide carrier to form a covalent linkage, preferably at a side chain of one or more of the peptide amino acid residues. For this purpose, amino acid residues having a nucleophilic side chain and having a reactive group such as amino (-NH₂), hydroxyl (-OH), or sulfhydryl (-SH) in its side chain will typically be used. Lysine and cysteine are especially useful for this type of conjugation, and therefore peptide carrier molecules including lysine or cysteine amino acid residues will be most preferred.

Generally, the compounds (II) of the invention may be prepared by reacting R-substituted catechol derivatives (formula (III)) with a peptide carrier under conditions that will lead to the formation of a covalent bond between the catechol molecule and one amino acid residue of the peptide. If the peptide includes more than one residue capable of forming a covalent linkage to the catechol compound, multiple substitutions on the peptide can be accomplished. As will be discussed in greater

detail below, the positioning of the substituent on the peptide and the number of substituents may have a significant effect on the potency of the conjugate. Preferred compounds according to the present invention will have one and at most two hapten-substituents.

- 5 The catechol compounds may be reacted at the unsubstituted ring carbons (positions a, b and c in formula (II)) by first oxidizing the compound, e.g., with silver oxide (Ag_2O), to produce a benzoquinone intermediate. Reaction of the intermediate with a cysteine-containing peptide generally leads to formation of a covalent bond at the ring position adjacent the R group (position c in formula (II)); reaction with a
10 lysine-containing peptide generally leads to substitution at the 5-carbon (position b) of the catechol ring. (See, e.g., Liberato et al. (1984) *J. Med. Chem.* 24:28-33).

- The carrier peptides haptenated according to the invention must bind to an MHC Class I or Class II molecule on antigen presenting cells. MHC Class II molecules are normally expressed only on B lymphocytes, macrophages, dendritic
15 cells, endothelial cells, and a few other cell types. An antigenic peptide binds to a MHC molecule within a cleft on the surface of the MHC molecule. The cleft on the surface of a MHC Class II molecule can accommodate peptides of various different lengths. Peptides eluted from mouse and human MHC Class II molecules average 15-18 amino acid residues in length. (Hunt et al. (1992) *Science* 256:1817; Chiczy et al.
20 (1993) *J. Exp. Med.* 178:27). MHC Class I molecules are generally present on all nucleated cells and present processed protein antigen to CD8+ cells (cytotoxic T cells). Class I molecules bind peptides of 8 to 9 amino acid residues in length and sometimes 10 to 11 amino acid residues in length (Hukzo et al, *J. Immunol.*, 151:2572) for presentation to antigen specific cytotoxic T cells.

- 25 Without wishing to be limited to any theory, it is believed that CD8+ as well as CD4+ cells are effectors contributing to DTH response. Thus MHC class I conjugate carriers which are capable of being recognized by CD8+ T cells as well as MHC class II conjugate carriers which are capable of being recognized by CD4+ T cells are useful in the methods of the present invention for targeting the various T
30 cells which are involved in the DTH response to urushiol compounds. However, it is believed that it is possible to regulate and modulate CD4+ and CD8+ concerted T cell response by precise targeting of the pertinent CD4+ T cell population alone, using only an MHC class II carrier conjugate in accordance with the invention (see, Examples 3, 4, and 5).

- 35 Peptides selected to serve as carriers for the haptenated peptides of the invention will comprise about 7 to 30 amino acid residues, preferably about 9 to 20 amino acid residues, more preferably 9 to 15 and most preferably 9 to 13 amino acid

residues. The carrier peptide must also contain at least one amino acid residue capable of reacting to form a covalent bond with a hapten (or reactive hapten intermediate). Lysine and cysteine are most preferred.

The present invention also includes the use of peptide-like molecules as carriers such as peptidomimetics which function in a similar manner to those peptides just described. Such peptidomimetics may incorporate unnatural amino acids, or may include modified linkages between consecutive residues. Replacement of peptide bonds with amide isosteres and transformation of the secondary structure of peptides into non-peptide molecules is also contemplated (see, Goodman and Seonggu
5 *Peptidomimetics for Drug Design: Burgers Medicinal Chemistry and Drug Discovery, 5th ed.: Volume I: Principles of Drug Design*, Manfred E. Wolff (ed.), pp 803-861 (January 1995) J. Wiley and Sons, New York). Peptidomimetics may be particularly suitable as a carrier in that they have more lasting effects in biological systems. For example peptides are subject to attack by enzymes in biological systems.
10 A peptidomimetic which includes amide bond isosteres resemble the amide bonds of conventional peptides but are more resistant to enzymatic cleavage *in vivo*. Peptidomimetics capable of binding class II MHC are described in the literature (Hill et al., *J. Immunol.*, 152:2890-2898 (March 1994). Specific peptidomimetics suitable as carriers in the instant invention are those which include non-peptide bonds and peptide bond analogs e.g. N-methyl amide bond ($\text{NH-C}_{\alpha 2}[-\text{CO-NCH}_3-\text{C}_{\alpha 1}]$) and reduced bond analogs ($\text{NH-C}_{\alpha 2}[-\text{CH}_2-\text{NH-}]\text{C}_{\alpha 1}$).
15

The individual amino acids of the carrier peptide may be characterized in one of three categories, depending on their relative positions within the peptide and their interaction with MHC molecules and TCRs: (1) several amino acids, usually 2 or 3
25 but usually not more than 7, will form an "agretope", which is a single unit of recognition that binds to one MHC molecule or one family of MHC molecules related by a consensus sequence; (2) several amino acids, usually 2 or 3 but usually not more than 7, will form, after haptentation, an "epitope", which is the basic element of recognition by a receptor, e.g., a T cell receptor; and (3) the remaining amino acids,
30 usually 4-10 amino acids, in addition to providing spacing for the agretopic and epitopic residues, also contribute to interaction of the peptide backbone with the MHC molecule are neither involved in binding to the MHC molecule nor to the T cell receptor, other than to provide spacing between the agretopic and epitopic amino acids. The amino acids making up the agretope may be separated or contiguous in the
35 sequence of the peptide. Likewise, the amino acids which together form the epitopic recognition sequence may be located together or separated by one to several, usually 1 to 4, amino acids. So long as binding to a MHC molecule is accomplished and at

least one site suitable for haptation is provided, the identity and exact sequence of the amino acids of the carrier peptide are not important.

In any particular MHC-binding peptide (e.g., a peptide of a known amino acid sequence that binds to a known MHC molecule), the amino acid positions within the peptide can be mapped as (1) MHC contact residues, (2) TCR contact residues or (3) "neutral" residues not directly involved in the MHC-binding or TCR recognition using standard techniques known to those skilled in the art. (See, e.g., Rothbard and Geftter (1991) *Ann. Rev. Immunol.* 9:527-565; and Jorgensen et al. (1992) *Ann. Rev. Immunol.* 10:835-873, incorporated herein by reference). For example, the amino acid residues of an antigenic peptide can be systematically substituted with other residues and the substituted peptides can then be tested in two different assays. In one assay, the ability of the substituted peptides (in excess) to compete with the wild-type peptide for binding to an MHC molecule on the surface of an antigen presenting cell is assessed. Alternatively, the ability of the substituted peptides to directly stimulate antigen-specific T cells is assessed. A peptide having an amino acid substitution at a position involved in binding of the peptide with the MHC molecule will exhibit altered binding capacity relative to the wild-type peptide. In contrast, a peptide having a substitution in an amino acid residue at a position involved in contact of the peptide with the T cell receptor will still be able to compete with the wild-type peptide for binding to the MHC molecule but will have altered ability to stimulate antigen-specific T cells (since the substituted peptide can still bind to the MHC molecule but is recognized differently by the T cell receptor). Finally, a neutral amino acid position can be substituted with many amino acids without significantly affecting the ability of the peptide to compete with the wild-type peptide for MHC binding and to directly stimulate antigen-specific T cells.

The ability of wild-type and substituted peptides to bind to MHC molecules can also be directly assessed using labeled peptides and purified MHC molecules in well known binding assays, such as equilibrium dialysis, column binding assays, and the like. (See, e.g., Sette (1987) *Nature* 328:395-399).

Several MHC-binding peptides suitable as carriers for haptens are known. They may be entirely synthetic or derived from the sequence of natural proteins, such as hen egg lysozyme (HEL), bovine or human serum albumin (BSA, HSA), ovalbumin (OVA), influenza hemagglutinin, and many others.

Although there are differences between and MHC class I and Class II binding molecules, there are also similarities (Rothbard, JB, *Curr. Biol.* 4:653-655 (Jul. 1994). Thus, with these similarities and differences in mind it is possible to choose or design a Class I carrier using a similar analysis as described below for the design or choice of

a Class II MHC binding carrier. For example Martin et al., *J. Immunol.*, 151:678-687 (Jul, 1993) describe a murine MHC class I H-2^b binding peptide which when conjugated to the hapten, TNP, are recognized by TNP hapten-specific T cells.

Examples of peptide carriers specific for Class II MHC includes a peptide of hen egg lysozyme (HEL) spanning amino acid residues 52-61 can be used. It is known that this peptide can bind to I-A^k and that the amino acid side chains at positions 53, 56 and 59 are T cell receptor contact residues. (See, Evavold et al. (1992) *J. Immunol.* 148:347-353). In the native HEL peptide, position 53 is a tyrosine residue, position 56 is a leucine residue and position 59 is an asparagine residue. Catechol compounds, such as PDC, or another hapten can be directly coupled to a lysine or other reactive amino acid substituted for leucine at position 56. The tyrosine at position 53 and the asparagine at position 59 can also be reacted to covalently bind to a hapten molecule or can be substituted with lysine or cysteine, and then reaction can take place more readily at the substituted amino acid.

An example of a peptide that binds to murine MHC Class II molecules and is suitable as a carrier peptide is a peptide in a murine system derived from the murine Class II MHC E α chain, spanning from residues 54-66. This peptide can bind to I-A^b or I-A^k (see binding data in Fig. 2) A residue at position 60 is a T cell receptor contact residue. Catechol compounds (III) or other haptens can be coupled to a lysine or other reactive amino acid substituted at position 60 for the native amino acid (leucine).

An example of a peptide for binding to a human MHC Class II molecule is a well-characterized peptide derived from influenza H3 hemagglutinin. The peptide encompasses amino acid residues 307-319 in the natural protein and has the following amino acid sequence:

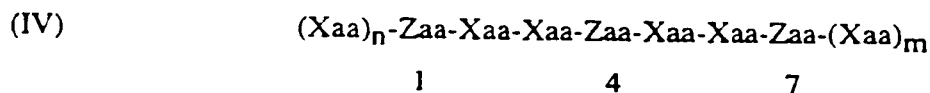
Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (SEQ. ID. NO. 53)

1	2	3	4	5	6	7	8	9	10	11	12	13
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This peptide is known to bind to the human MHC Class II molecules HLA-DR1 and HLA-DR4 with an affinity in the low nanomolar range. T cell receptor contact residues have been mapped to positions 308 (position 2 above), 310 (position 4 above), 311 (position 5 above), 313 (position 7 above) and 316 (position 10 above) by substitution analysis (Krieger et al. (1991) *J. Immunol.* 146:2331-2340) and from crystallographic studies (Brown et al. (1993) *Nature* 364:33-39). The amino acid residues at positions 308, 311 and 316 are lysines.

Therefore, catechol compounds (or other haptens) can be advantageously coupled to the side chain of one or more of the lysines at the these positions by standard techniques. Additionally, the valine residue at position 310 can be replaced with an amino acid residue having a reactive side chain, preferably lysine or cysteine, and a catechol compound can be coupled to the substituted amino acid residue. Similarly, the asparagine residue at position 313 can be reacted with a catechol compound to haptenate the peptide, or it can be replaced with another reactive amino acid, preferably lysine or cysteine, and a catechol compound can be coupled to the substituted amino acid residue.

Based on prior studies of the structures of antigenic peptides and investigation on peptide requirements for MHC class II binding (Rothbard et al., *Int. Arch. Allergy Immunol.*, 105:1-7 (Sep. 1994)), a general structure for preferred carrier peptides can be discussed. The most preferred carriers for Class II MHC binding will be 9 to 13 amino acids in length, and the amino acid residues that are the targets for linking to a hapten molecule will be spaced at specific relative positions within the molecule. It is believed that these particular positions within a peptide are preferentially recognized (i.e., contacted by) the T cell receptor on antigen-specific T lymphocytes regardless of which protein the antigenic peptide is derived from. It is at these positions that hapten molecules, e.g., catechol compounds, may be most advantageously bound. The pattern of the amino acid residues in such preferred MHC Class II-binding carrier peptides can be represented as follows:



In the above motif, Xaa and Zaa are amino acid residues, and Zaa represents a target residue for hapten linking. One or more of the Zaa amino acids, therefore, will have a reactive side chain and will most preferably be a lysine or cysteine residue. The Xaa amino acids are selected independently and can each be different from the other. It is most preferred that the Xaa amino acid at position 3 be a phenylalanine, tyrosine or isoleucine. The designations n and m represent integers from 1 to 3, with n + m equaling 2 to 6.

While the above structure and the values for n and m provide an optimal peptide for MHC binding, much larger peptides containing this motif (i.e., n and/or m is a larger integer, e.g., up to 30 or more) could also readily bind to MHC molecules. It should be recognized, therefore, that in synthesizing or selecting the carrier peptide including the above structure (IV) that many additional residues can be added to the

amino terminal and/or carboxy terminal ends without detrimentally affecting MHC binding. Indeed, addition of more amino acid, such as polyalanine tails, may be desirable to secure advantages unrelated to MHC binding, for example increasing the serum half-life of the compound.

- 5 As indicated above, the Zaa amino acids are selected to provide reaction sites for the hapten molecules while having a position within the MHC-binding carrier peptide which places them in the optimal position for presentation to the receptor complexes of T lymphocytes. Any position except for position 3 are potentially suitable for reaction sites for the hapten molecule. However preferred positions
10 include positions 4, 7, 9, and 11. The most preferred Zaa position along the MHC binding carrier peptide is position 7 (see Examples). The Xaa amino acids are selected to provide suitable residues for binding to the MHC Class II antigen (i.e., agretopic residues) and residues for maintaining the spatial relationship of the other residues involved in either MHC binding or T cell receptor presentation. The precise
15 combination of Xaa residues employed depends in part on the specific MHC allele to which the MHC-binding peptide is desired to bind. For many antigenic peptides of known sequence, the identity of amino acid residues that are necessary for MHC binding has been determined. (See, e.g., Rothbard and Geftter (1991) *Ann. Rev. Immunol.* 9:527; Evavold et al. (1992) *J. Immunol.* 148:347; Krieger et al. (1991) *J. Immunol.* 146:2331; and Sette et al. (1993) *J. Immunol.* 151:3163). In addition, for
20 any peptide of a known amino acid sequence which binds to an MHC molecule, one skilled in the art can map which amino acid residues at which amino acid positions are necessary for binding of the peptide to an MHC molecule and which amino acid positions can accommodate substitution with other amino acids (see, e.g. Alexander et al., *Immunity*, 1:751-761 (1994)).
25

Especially preferred peptides according to the above formula (IV) will have $n = 3$ and $m = 1, 2$ or 3 . Lysine is most preferred for one or more of the Zaa positions, and tyrosine, isoleucine or phenylalanine will preferably be at position 3. As will be discussed below, all the other residues may advantageously be alanine residues.

- 30 For example, further studies on the influenza H3 hemagglutinin peptide described above have been performed to determine which amino acid residues are important for binding to HLA-DR1. It was found that a synthetic peptide containing only two residues from the native hemagglutinin peptide embedded within a chain of polyalanines retained the ability to bind to HLA-DR1 (Jardetzky et al. (1990) *EMBO J.* 9:1797-1803). Thus, most of the amino acids within the peptide can be substituted,
35 e.g., with alanine residues. The synthetic HLA-DR1-binding peptide comprises an amino acid sequence:

Ala-Ala-Tyr-Ala-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala (SEQ. ID. NO. 54)

1 2 3 4 5 6 7 8 9 10 11 12 13

- 5 This synthetic peptide can also be used as a carrier for catechol compounds or other haptens to produce haptenated peptides according to this invention. For example, the lysine at position 10 above can be haptenated. Furthermore, the alanines at T cell contact positions, e.g., positions 4 and 7 in the above formula, can be substituted with amino acids having reactive side chains, preferably lysine or cysteine, to provide
- 10 additional haptenation sites along the peptide. Particularly preferred carrier peptides, therefore, which can be readily haptenated with catechol compounds and which bind to a human MHC Class II molecule (e.g., HLA-DR1) have the following amino acid sequence:

15 Ala-Ala-Tyr-Zaa-Ala-Ala-Zaa-Ala-Ala-Lys-Ala-Ala-Ala (SEQ. ID. NO. 55)

1 2 3 4 5 6 7 8 9 10 11 12 13

wherein Zaa is alanine or an amino acid residue having a nucleophilic side chain but most preferably lysine or cysteine.

- 20 Another synthetic polyalanine-based peptide which retains the ability to bind to human MHC Class II molecules has similarly been defined and can be used as a hapten carrier. This peptide has the amino acid sequence:

Ala-Ala-Ile-Zaa-Ala-Ala-Zaa-Ser-Ala-Zaa-Ala-Ala-Ala (SEQ. ID. NO. 56)

25 1 2 3 4 5 6 7 8 9 10 11 12 13

- wherein Zaa is alanine or an amino acid residue having a nucleophilic side chain, preferably lysine or cysteine. Catechol compounds or other haptens can be coupled to the peptide at T cell receptor contact residues, e.g., at positions 4, 7 and/or 10 of the
- 30 peptide (most preferably position 7), to produce haptenated peptide compounds according to this invention.

- It is further predicted that peptides can be designed which will bind to many different haplotypes of a MHC molecule. For example, previous studies have shown that the same "promiscuous" peptide sequence can be eluted from
- 35 DR2, DR3, DR4, DR7, and DR8 molecules. (See, Chicz et al. (1993) *J. Exp. Med.* 78:27-47). These "promiscuous" peptide sequences can be used as the basis for designing haptenated peptides which can bind to many different MHC

haplotypes (i.e., "universal binders"), using techniques discussed above. Peptides identified as "universal" or "nearly universal" binders are discussed in Marshall et al. *J. Immunol.* 152:4946-4947 (May 1994) and in Alexander et al., *supra*. A single haptenated peptide based on the polyalanine backbone discussed above will bind to almost all DR alleles at an affinity below 200 nM. Use of a single carrier peptide, particularly a universal binder, as a hapten carrier would eliminate major disadvantages of prior methods for treating urushiol contact sensitivity that employ autologous cell membranes as carriers: Use of a single peptide as a substrate for the hapten would permit formulation of uniform preparations and would avoid the use of blood products, which are susceptible to contamination, unsuitable for general administration and complicated to formulate due to the need for maintaining sterility at all manufacturing stages. The haptenated peptides of the invention have a further advantage in that they are addressed directly to the cells of the immune system, i.e., they are intended to bind directly to Class II-bearing APCs, and thus are believed to more efficiently bring about their effects on the immune system, e.g., in that more of the administered dose of the compound will be targeted to the specific cell populations involved in the hapten-specific hypersensitivity.

Preferred carrier peptides which have been modified or designed in accordance with the invention to bind many different MHC haplotypes using formulas and techniques discussed above include, but are not limited to, the following peptides TTK (SEQ. ID. NO. 1), TTK:7 (SEQ. ID. NO. 2), C03 (SEQ. ID. NO. 3), C03:7 (SEQ. ID. NO. 4), DR002:0 (SEQ. ID. NO. 5), DR002:7 (SEQ. ID. NO. 6), DR003:0 (SEQ. ID. NO. 7), DR003:7 (SEQ. ID. NO. 8), DR004:0 (SEQ. ID. NO. 9), DR004:7 (SEQ. ID. NO. 10), DR005:0 (SEQ. ID. NO. 11), DR005:10 (SEQ. ID. NO. 19), DR005:11 (SEQ. ID. NO. 20), DR005:12 (SEQ. ID. NO. 21), DR005:2 (SEQ. ID. NO. 12), DR005:4 (SEQ. ID. NO. 13), DR005:5 (SEQ. ID. NO. 14), DR005:6 (SEQ. ID. NO. 15), DR 005:7 (SEQ. ID. NO. 16), DR005:8 (SEQ. ID. NO. 17), DR005:9 (SEQ. ID. NO. 18), DR006:0 (SEQ. ID. NO. 22), 006:7 (SEQ. ID. NO. 23), 007:0 (SEQ. ID. NO. 24), 007:7 (SEQ. ID. NO. 25), 008:0 (SEQ. ID. NO. 26), DR009:0 (SEQ. ID. NO. 27), DR010:0 (SEQ. ID. NO. 28), DR011:0 (SEQ. ID. NO. 29), DR011.7 (SEQ. ID. NO. 30), DR012:0 (SEQ. ID. NO. 31), DR013:0 (SEQ. ID. NO. 32), DR014:0 (SEQ. ID. NO. 33), HLA001:0 (SEQ. ID. NO. 34), HLA001:4 (SEQ. ID. NO. 35), HLA001:5 (SEQ. ID. NO. 36), HLA001:6 (SEQ. ID. NO. 37) all as shown in Fig. 1. Preferred peptides include DR005:7 (SEQ. ID. NO. 16), DR011:7 (SEQ. ID. NO. 30), TTK:7 (SEQ. ID. NO. 2), and C03:7 (SEQ. ID. NO. 4) all as shown in Fig. 1. Of the above peptides, those peptides indicated by peptide #:0 (e.g

5 Additionally, peptidomimetics useful in accordance with the present invention may be based on any of the above peptides or "parent" peptides.. For example, a peptidomimetic based on DR005:0 (SEQ. ID. NO. 11) may have the following structure:

wherein [-] is a normal peptide bond, [*] is an N-Methyl amide bond or a normal peptide bond and [\$] is a reduced bond analog or a normal peptide bond. It is contemplated that a peptidomimetic may include one or more [*] or[\$] bonds or various combinations thereof.

In preparing the catechol derivative-substituted peptides of formula (II), it may be desirable to decrease the hydrophobicity of the hydrocarbon side chain (R). Data suggests that the hydrocarbon side chain at position 3 of the catechol nucleus of urushiol is important in adherence and entry into the skin, while the catechol head group is responsible for haptenization of endogenous proteins. (See, Baer et al. (1967) *J. Immunol.* 99:370-375). This lipid side chain can be truncated so as to be much less hydrophobic and more highly soluble in aqueous environments. Such compounds may also have decreased toxicity as compared to the natural urushiol, since the natural toxicity of urushiol is associated with the ability of the long, lipophilic side chain to interact with cell membrane lipids. Catechol compounds with altered hydrocarbon side chains can still be recognized by urushiol-specific T lymphocytes. Thus, the use of substituted catechol compounds (III) having R

substituents of, e.g., ten carbons or fewer will yield haptenated peptides exhibiting enhanced properties (e.g., increased solubility, decreased toxicity) without eliminating the ability of the hapten to be recognized by hapten-specific T cells. Other modifications which may be made to increase solubility include modification with polyethylene glycol (PEG) using the method of Wie et al., *Int. Arch. Allergy and Appl. Immunol.*, **64**:84-99 (1981), to produce a peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a peptide of the invention. Solubility may also be enhanced by including various chemical groups at the amino terminus of the peptide during peptide synthesis or by chemical reaction after synthesis. Such chemical groups include but are not limited to: sulfoacetyl, phosphonoacetyl, lactobionyl, and carboxymethyl-polyethyleneglycol-methoxy ethers of varying lengths.

As discussed earlier, the present invention also contemplates the use of a non-peptide carrier which may be bound to a non-MHC encoded molecule capable of presenting antigen to T cells. Specifically, the carrier may be a lipid which can bind to certain classes of CD1 molecules which specialize in presenting certain lipid antigens to cytotoxic T cells. Recently, an antigen presentation function has been proposed for CD1 molecules, a family of non-MHC-encoded, non-polymorphic, β -2 microglobin-associated glycoproteins found on most professional antigen presenting cells (reviewed by Parham, Peter, *Nature*, **372**:615-616 (December 15, 1994)), Beckman et al, *Nature*, **372**:691-694 (1994) recently report that the CD1b molecule presented a lipid antigen of *Mycobacterium tuberculosis* to human $\alpha\beta$ T cells. Beckman et al, demonstrated that, unlike MHC Class I and Class II molecules, the antigen presented by CD1b was not a peptide, but rather a long-chain fatty acid. This demonstrated that human T cells can recognize antigen of a totally non-protein nature, and secondly, that lipid antigens can be presented by a specific molecule found on antigen presenting cells.

In view of this, it is possible that CD1b molecules participate in the T cell recognition of urushiol for the following reasons. First urushiol is a family of compounds that possess 15-17 carbon-long sidechains that impart lipophilic properties to the molecules (reviewed in Tyman, Chem. Soc. Rev. **8**:499-537, (1979). Second, CD1b molecules are found on Langerhans cells, the major antigen-presenting cells of the human skin (Knapp et al. (eds.), *Leucocyte Typing IV. White Cell differentiation Antigens*, Oxford University Press, (1989)). Finally, although T cell responses to urushiol include conventionally-restricted CD4+ and CD8+ T cells (Kalish, *J. Invest. Dermatol.* **90**:108s-111s (1990)), additional T cell responses to CD1b-presented lipids cannot be ruled out. CD1b molecules may either present

urushiol directly by the lipid portion of the molecule, or alternatively, the urushiol may couple to other lipids that would then be presented by CD1b. To test this, the CD1b molecule can be transfected into cells that lack MHC class I or class II antigens, as reported in Fig. 4 of Beckman et al, *supra*. These cells can then be
5 exposed to either purified urushiol, or to lipids or lipid extracts from cells that had previously been reacted with urushiol. Populations of T cells from urushiol-sensitive individuals could then be tested for the ability to recognize the urushiol-exposed versus unexposed CD1b-transfected antigen-presenting cells. Verification of this specificity would require selection of a CD1b-restricted T cell line or clone as done
10 by Beckman et al., *supra*. Such T cells may have a significant role in hypersensitivity to urushiol.

Thus, a CD1b binding lipid carrier may be used as a carrier molecule for conjugation to a catechol (formula III) in accordance with the invention. Such a lipid-carrier haptenated molecule, when administered to a hypersensitive individual
15 as described below, may be capable of participating in the desensitization of individuals suffering from hypersensitivity to the contact allergen.

The haptenated compounds of the invention can be administered alone or incorporated into a composition comprising a haptenated compound and a pharmaceutically acceptable carrier or diluent. The term "pharmaceutically
20 acceptable carrier or diluent" is intended to include substances capable of being co-administered with the active ingredient (haptenated MHC-binding peptide) and which will not hinder its activity. Examples of such carriers and diluents include sterile buffered saline solutions, solvents, dispersion media, delay agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in
25 the art. Any conventional agent compatible with the haptenated peptide can be used with this invention.

The haptenated compounds of the invention are useful in the treatment of contact sensitivity, e.g., to desensitize a hypersensitive individual against exposure to a contact allergen. The haptenated peptide compounds of the invention can also be
30 used to desensitize hapten-sensitized mammals, including humans. The term "hapten-sensitized" is used to refer to an individual susceptible to a hapten-specific sensitivity and who has been previously exposed to the hapten. Desensitization of a hapten-sensitized individual means that the hapten-sensitized individual will not produce a hapten-specific DTH response upon exposure to the hapten or will produce
35 a response of diminished intensity to untreated subjects. Such hapten-specific contact sensitivity reactions are well understood and can be visually evaluated, e.g., using the Draize system. (See, U.S. Patent No. 4,428,965). Furthermore, Example 2G

describes murine desensitization experiments using haptenated compounds of the invention.

The haptenated peptides described above may be used to treat contact sensitivity to environmental haptens. Specifically, the catechol compound-substituted peptides described above can be used to treat contact sensitivity to urushiol. A urushiol-sensitized mammal can be desensitized to the urushiol contact allergen by administration of a haptenated peptide in non-immunogenic form. It is believed that the haptenated peptides, when administered in non-immunogenic form induce hapten-specific non-responsiveness by causing hapten-specific T cells to become nonresponsive to the hapten. The ability to induce antigen-specific T cell nonresponsiveness in a subject by administering to the subject a peptide derived from the antigen has been documented both with antigens involved in allergic responses (e.g., *Fel d I*, see Briner et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:7608-7612) and in autoimmune diseases (e.g., experimental autoimmune encephalomyelitis; see Smilek (1991) *Proc. Natl. Acad. Sci. USA* 88:9633-9637 and Wraith (1989) *Cell* 59:247-255). The invention further encompasses a composition for inducing hapten-specific T cell nonresponsiveness in a subject comprising at least one haptenated peptide as described previously. In addition, compositions of the instant invention may include one or more haptenated peptides of the invention. Such compositions may be administered simultaneously or sequentially. In order to maximize the down-regulating effect on hapten-specific T cells, it may be advantageous to administer several haptenated molecules specific for each class of hapten-specific T cell in a single composition or multiple compositions which can be administered simultaneously or sequentially. For example, one haptenated molecule may comprise an MHC Class I binding peptide, another haptenated molecule may comprise an MHC Class II binding peptide and yet another haptenated molecule may comprise a CD1b-binding lipid. In this manner a range of T cells which may participate in the hypersensitive response to urushiol (e.g. cytotoxic T cells which recognize a peptide bound to a Class I MHC, helper T cells which recognize peptide bound to Class II MHC, and cytotoxic T cells which recognize lipid bound to CD1b) may be targeted for down regulation thereby enhancing the desensitizing effect of the compounds of the invention.

The haptenated molecules of the present invention can be administered in a convenient manner such as by injection (subcutaneous, intradermal, intravenous, intraperitoneally, etc.), oral, nasal sublingual or rectal administration, or by inhalation. Preferred routes of administration are intravenous injection and subcutaneous injection. Depending on the route of administration, the peptide can be incorporated

into a material to protect it from the natural conditions which may detrimentally affect its ability to perform its intended function, such as action by enzymes, acids, and other natural conditions which detrimentally affect the peptides. Examples of suitable carriers include but are not limited to physiological saline for injectable forms; starch, sucrose, lactose, gelatin, magnesium stearate, acacia for oral dosage forms (solid); water and edible oils such as peanut oil for oral dosage forms (liquid). The haptenated peptide compound is administered in a non-toxic dosage concentration sufficient to induce T cell non-responsiveness or to desensitize the subject to the allergenic effects of the contact allergen, e.g., urushiol. The actual dosage will be determined by recognized factors such as body weight, age, severity of the allergic reaction, potency or activity of the particular active compound to be administered, factors peculiar to the individual patient such as sensitivity to other medications, and other such factors familiar to those who practice in this area. An effective dosage and the desensitizing effect can be readily determined by the medical practitioner in accordance with conventional techniques in the medical art.

The present invention is further illustrated by the following examples which should in no way be construed as being further limiting. The contents of all cited publications not previously incorporated are hereby expressly incorporated by reference. All amino acid sequences referred to herein are written in N-terminal to C-terminal order.

EXAMPLE 1: T cells specific for PDC respond to PDC-substituted carrier peptides

3-*n*-pentadecyl catechol (PDC) is a major component of poison ivy urushiol and is capable of inducing and eliciting delayed-type hypersensitivity rashes. (ElSohly et al. (1982) *J. Nat. Prod.* 45: 532-538; Baer et al. (1967) *J. Immunol.* 99: 370-375).

Synthesis of pentadecyl catechol

Synthesis of 3-*n*-pentadecyl catechol (PDC) was carried out according to the procedure described by Dawson and Ng in which veratrole (1,2-dimethoxybenzene) was first metalated using *n*-butyl lithium and reacted with 1-bromopentadecane to form 3-*n*-pentadecyl veratrole (3-PDV). (See, Dawson and Ng (1978) *Org. Prep. Proc. Int.* 10:167-172). Boron tribromide was then used to cleave the methyl ether groups of PDV, forming 3-PDC. A noteworthy modification in the present protocol was the use of flash chromatography in purification of the 3-PDV and 3-PDC instead

of vacuum distillation. Structures of both 3-PDV and 3-PDC were confirmed by melting point, ^1H NMR and FAB-MS. The newly prepared 3-PDC had an R_f in TLC analyses identical to an authentic sample obtained from the Bureau of Biologics, FDA.

5

Coupling PDC to peptides and proteins

The regiospecificity and reactivity of PDC coupling to protein carriers has been studied previously. (Liberato et al. (1984) *J. Med. Chem.* 24: 28-33). In general, full-length proteins and a peptide were coupled to PDC covalently through either the ϵ -amino group of lysine or the sulfhydryl group of cysteine by first converting the PDC to the o-benzoquinone using Ag_2O as an oxidizing agent in THF. The freshly prepared o-benzoquinone solution was filtered directly into the peptide solution in dimethylsulfoxide (DMSO) with stirring. After the removal of the reaction solvent, the residue was taken up in water and extracted with ethyl ether to remove the excess PDC. The PDC-substituted products were further purified by silica gel chromatography using chloroform:ethanol:water as the solvent system. The structure of the purified products were confirmed by amino acid analysis and mass spectroscopy.

The carrier proteins, mouse IgG (ATCC accession no. HB183), hen egg lysozyme (HEL, Sigma Chemical, St. Louis MO), or ovalbumin (OVA, Sigma Chemical, St. Louis MO) (in range of 7-140 nanomoles) were reacted with excess PDC (16 μmol) in pure DMSO at room temperature using air oxidation of the catechol. The reaction was monitored spectrophotometrically; a characteristic absorbance peak at about 480 nm developed as expected. (Liberato et al. (1984) *J. Med. Chem.* 24: 28-33). PDC was bound to an MHC-binding peptide from murine E α protein, residues 54-66 (FEAQGALANIAVD (SEQ. ID. NO. 43) in single-letter code), following the same procedure, after substitution of a lysine for the leucine at position 60 (FEAQGAKANIAVD) (SEQ. ID. NO. 45).

A monoclonal antibody was prepared against PDC by standard procedures: Balb/c mice were immunized with PDC-coupled IgG in Ribi adjuvant. Splenocytes were fused with the SP2/O myeloma and hybridomas selected in HAT medium. A monoclonal IgM antibody was identified which recognized PDC-substituted but not unsubstituted IgG.

The anti-PDC monoclonal antibody was used in immunoassays of the PDC-carrier preparations. The results showed that the anti-PDC antibody showed binding to the PDC-substituted mouse IgG, but not the unsubstituted (control) mouse IgG (data not shown). PDC-substituted proteins were precipitated and extracted with

methanol, lyophilized and digested with trypsin before use in T-cell assays. These treatments were found to remove toxicity in cellular assays, most likely due to residual PDC, and to increase the solubility of the preparations.

5 Murine T cell responses to PDC-peptides

The ability of Class II MHC-restricted, PDC-specific T cell clones to recognize an arbitrary peptide coupled with PDC was examined. T-cell hybridoma clones were produced by fusing T cells derived from C3H mice that had been immunized with either HEL-PDC or murine IgG-PDC, and restimulated three days in
10 culture with the antigens HEL-PDC or HEL-catechol before fusion. Resulting T hybridomas were screened for their ability to secrete interleukin-2 (IL-2) in response to syngeneic APCs (CH27, see, e.g., Pennell et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:3799-3803) and HEL-PDC or HEL-catechol. Two PDC-specific T hybridomas were cloned: 9/29 F6 and 3.15.

15 The two independently-derived I-A^k-restricted hybridoma clones were then tested for their ability to recognize an "arbitrary" peptide coupled with PDC (i.e., a peptide carrier to which the T cell hybridomas had not been previously exposed). The peptide used for restimulation was derived from the MHC Class II E α chain, amino acid residues 54-66. This peptide is known to bind to I-A^b (Rudensky et al. (1992) *Nature* 359:429-31) and has now been discovered to bind to I-A^k.

T hybridoma 9/29 F6 was assayed for IL-2 production in the presence of APCs and PDC-substituted or unsubstituted E α peptide. Peptides in two-fold serial dilutions were added to cultures containing CH27 cells as APCs. T cells were then added and 24-hour supernatants tested for IL-2 using HT-2 indicator cells (murine
25 helper T cell line, ATCC accession no. CRL1841). Quantitation was performed using the Alamar® Blue dye assay kit (Alamar, Sacramento CA). The data, illustrated in Figure 3, show that the PDC-substituted, lysine-modified peptide E α ₅₄₋₆₆ (K60:PDC-E α , having the amino acid sequence: FEAQGAK(PDC)ANIAVD (SEQ. ID. NO. 45), with PDC bound to the lysine residue substituted at position 60) was
30 recognized (in a dose-dependent manner) by the 9/29 F6, PDC-specific T hybridoma cultured with various concentrations of the K60:PDC-E α peptide. The PDC-specific T hybridomas did not recognize the unhaptenated control E α peptide (FEAQGAKANIAVD) (SEQ. ID. NO. 45).

T hybridomas that were specific for PDC (3.15) and for HEL (11.6) were
35 assayed for IL-2 production in the presence of CH27 APCs pulsed with PDC-substituted E α peptide, control (unsubstituted) E α peptide, or full length HEL protein. CH27 cells were incubated with 4 μ g/ml of either control E α peptide or K60:PDC-E α

peptide, or HEL protein at 20 µg/ml, for 16-20 hours. The antigen-exposed CH27 cells were washed and diluted in two-fold steps from 1.2×10^5 cells/culture. T hybridoma cells (5×10^4 cells/culture) specific for PDC (3.15) or specific for HEL (11.6) were added to the cultures and after 24 hours supernatants were collected and
5 analyzed for IL-2 release using HT-2 cells and Alamar® Blue dye.

The results (data not shown) indicated that APCs pulsed with the K60:PDC-E α peptide were recognized by the PDC-specific 3.15 hybridoma, but not by the HEL-specific, I-A^k restricted 11.6 hybridoma. Neither hybridoma recognized the unhaptenated control Eα peptide; and APCs pulsed with HEL protein were
10 recognized only by the 11.6 hybridoma. These results establish that PDC as a hapten can be recognized by T cells when coupled to an arbitrary peptide of appropriate MHC binding capability.

15 EXAMPLE 2: Hapten-specific down regulation of DTH response induced by PDC in a murine model

The purpose of the following experiments is to study MHC class II restricted T cell responses to urushiol haptens in a mouse model with an H-2^k MHC background. Synthetically prepared 3-pentadecyl-catechol (PDC) which contains a
20 saturated 15 carbon sidechain and is a major component of urushiol, was used as the hapten in these studies. Liberato et al. *J. Med. Chem.* 24:PDC is known to couple to peptides and proteins specifically through cysteine and lysine residues. The following experiments specifically address the position of the hapten on the carrier peptide, the cross-reactivity of synthetic hapten conjugates with t cells induced from skin painting
25 with the hapten alone, and whether or not such synthetic compounds can down-regulate *in vivo* DTH responses induced by cutaneous exposure to the free hapten.

PROCEDURES:

30 Molecular Modeling

A model of the binding of peptides to the I-A^k molecule was developed based on the crystal structure of the HLA-DR1 molecule containing the hemagglutinin peptide HA 307-319 (Wiley, Stominger et al.,) using the QUANTA modeling package from Molecular Simulations, Inc

35

Peptide Synthesis:

Peptides were synthesized employing solid phase techniques, either using an Applied Biosystems Peptide synthesizer or an Advanced Chemtech robotics system utilizing FxtMOC_{TM} chemistry with commercially available Wang resins, and Fmoc protected amino acids, as previously described. Fig. 2 lists the names and sequences of the peptides used in this study. For synthetic ease, all of the peptides used were acetylated at the amino terminus and were amidated at the carboxy terminus. All peptides were purified using reverse phase HPLC and their structures were confirmed by amino acid analysis and fast atom bombardment mass spectrometry.

10 Synthesis of TNP-Coupled Peptides:

The synthesis of these conjugates was done while the peptides were still coupled to the solid phase support using standard protocols Good et al., *Selected Methods in Cellular Immunology*, B Mishell and S.M. Shiigi (eds), p 343 (New York, 1980).

15 Synthesis of PDC-Coupled Peptides

A quantity of 0.03 mmol of the desired carrier peptide was dissolved in dry DMSO:DMF (1:5 v:v, approximately 10ml.). While the peptide was dissolving, a stock solution of PDC converted to its quinone form was prepared by dissolving 0.05 mmol of PDC in 2.5 ml of MeCN, followed by the addition of 1.35 mg of powdered AgO. This mixture was swirled for 25 minutes, then filtered through a 0.1 μ m PTGE filter directly into the peptide solution. The resulting mixture was agitated (rocked) for 4 hours at room temperature.

After the agitation was stopped, the excess PDC/quinone was removed by the slow addition of 20 ml of dry diethyl ether (added in 1.0 ml aliquots with swirling) over the course of one hour. The resulting precipitate was separated from the ether solution, washed twice with ether, and dissolved in 50°C distilled water. After the aqueous solution had cooled, it was extracted twice with dichloromethane. Then the aqueous solution was dried (rotary evaporation) and was purified by reverse phase chromatography (C-18 HEMA cartridges, Alltech) using either acidic (TFA-water-acetonitrile) or basic (diisopropylethylamine-water-acetonitrile) solvent systems, and the purity of the material was assessed by mass spectroscopy. Final yields ranged from 28% to 79% based on starting peptide.

35 Purification of I-A^k Proteins

The procedures used for detergent solubilized and affinity purification of H-2-I-A^k molecules were similar to those described by Babbitt et al., *Nature*, 317:359

- (1985). Briefly, the B lymphoblastoid cell line CH27, which expresses I-A^k α and β chains, was grown in RPMI 1640 medium supplemented with 10% heat inactivated FCS, 2 mM glutamine, and antibiotics. Cells were harvested, washed in PBS, lysed with 1% NP-40 and the supernatant separated from nuclear debris by centrifugation.
- 5 The solubilized MHC class II proteins were affinity purified using the monoclonal antibody, 10.3.6.2 (28), coupled to Sepharose, after being passed through a Sepharose CL-4B precolumn. MHC class II proteins were eluted with 1% octyl- β -D-glucopyranoside (octyl glucoside), 50 mM phosphate at pH 11.5, and immediately neutralized using 1 M phosphate at pH 6.0, then concentrated to approximately 1
- 10 mg/ml using an Amicon stirred cell concentrator. Finally, the concentrated protein solution was fractionated by gel filtration in PBS + 1.0 % octyl glucoside + 0.2% NaN₃ using a 75 x 2.5 column of BioGel A0.5 (BioRad), and collecting the fractions that corresponded to the molecular weight of the $\alpha\beta$ -heterodimer (nominal 60 kD). The purity of the final material was assayed by SDS PAGE, HPLC gel filtration, and
- 15 Edman sequencing.

MHC Class II Peptide Binding and Inhibition Assays:

- For direct binding assays, an optimal concentration of affinity purified I-A^k(35 nM) was incubated with serial dilutions of a peptide HADP7.47,
- 20 AAYKAAKAAAAA (SEQ. ID. NO. 38), modified with long chain biotin at the amino terminus (NLCB:HADP7.47) in PBS containing 0.02% dodecyl maltoside at pH 5.5, in 96 well polypropylene plates (Costar) for 16-20 hours at 37°C. In studies optimizing the assay, between 5 to 10% of the I-A^k molecules were capable of binding added peptide. Therefore the effective concentration of I-A^k was
- 25 approximately 3.5 nM. The conditions of the assays were shown to be in ligand excess, because a two-fold reduction (of these class II molecules) did not change the measured ED₅₀ values. The I-A^k-peptide complexes (50 μ l) were transferred, in duplicate, to wells of a 96-well microtiter plate precoated with the monoclonal antibody 10.3.6.2 and blocked with heat inactivated fetal calf serum. An additional
- 30 50 μ l of 50 mM TRIS pH 7.0 containing 0.02% Tween 20 and 0.05% NaN₃. Europium labeled streptavidin (Pharmacia) was added and incubated overnight. After washing, complexes were measured by the addition of 0.1 M acetate/phthalate buffer, pH 3.2, containing 0.1% Triton X-100, 15 μ M 2-naphthoyltrifluoroacetone and 50 μ M tri-N-octylphosphine oxide, which released the chelated europium from streptavidin
- 35 and formed a highly fluorescent micellar solution. The resultant fluorescence was measured using a fluorescent plate reader (DELPHIA, LKB/Pharmacia). The data

was analyzed using a fitting algorithm to a theoretical binding equation that calculated the concentration of peptide giving a half-maximal signal (ED_{50}).

The inhibition assay format was identical to the procedure described above with the exception that the unlabeled peptide antagonist was serially diluted and incubated with constant concentrations of NLCH:HADP7.47 and the I-A^k protein. The concentration of unlabeled peptide that prevented 50% of the labeled peptide from binding was the IC_{50} value. The concentration of the NLCH:HADP7.47 used in each assay was experimentally determined to be at least one sixth of its measured ED_{50} in order to assure the inhibition as primarily measuring the binding characteristics of the competitor peptide. This was confirmed by comparing the IC_{50} of unlabeled HADP7.47 (SEQ. ID. NO. 38) to the measured ED_{50} of the labeled peptide, demonstrating that the IC_{50} solely measured the affinity of the antagonist for the receptor and was independent of the presence of the agonist. However, the IC_{50} and ED_{50} values are referred to being equivalent to the $K_D^{apparent}$ and not a true K_D , because of the known decomposition of MHC class II molecules during the course of the assay (Sette et al., *J. Immunol.*, **148**:844 (1992); Barany and Merrifield, *The Peptides*, E. Gross and J. Meienhofer, eds. Academic Press, New York). Therefore, the incubation time was chosen to permit sufficient amount of complex to be formed to allow ease of detection, but was minimized to limit the amount of decomposition of the receptor. The presence of endogenous peptides makes the assay an exchange reaction, rather than a simple binding event. Consequently, the inherent characteristics of the MHC molecules prevent the assay from ever attaining true equilibrium. However, by balancing the time of incubation, the $K_D^{apparent}$ can approximate the true K_D .

Animals:

C3H/HeN/HeN [K^k -IA^k I-E^k D^k] mice were purchased from Simonsen Labs (Gilroy, CA.). B10.A(5R) [K^k -A^k D^b], AT.L [K^s I-A^{s/k} I-E^k D^d], C57B1/6J [K^b I-A^b D^b], B10.A(5R) [K^b I-A^b I-E^{b/d} D^d], PLJ/SJL [K^{ws} I-A^{ws} I-E^{ws} D^{ws}] mice were purchased from Jackson Labs (Bar Harbor, Maine).

Cells were obtained from draining inguinal or popliteal lymph nodes (LN) of variously treated mice. Syngeneic spleen cells [irradiated 3000 rads and lysed with Red Blood Cell Lysing Solution (SIGMA)] were used as antigen presenting cells (APCs). Syngeneic spleen cells (prepared as above) were hapten conjugated at room temperature for 30 minutes by exposure to 20 mM purified PDC, purified urushiol, or TNP chloride. Following extensive washing with PBS, the hapten conjugated preparations were used as a source of antigen.

T cell cultures were supplemented with recombinant mouse IL-2 (40 units/ml from Genzyme, Cambridge, MA) after 3 to 4 days and every 2 to 3 days thereafter. Cultures were re-stimulated with fresh APCs and antigen every 10 to 14 days.

5 T cell Activation Assays:

T cells (2×10^4 per well) and APCs ($5 \cdot 10^5$ per well) were incubated in 96 well microtiter plates with a panel of antigens and appropriate controls for 72 hours. ^3H thymidine (0.5 mCi/well) was added for an additional 6 to 8 hours of incubation before harvesting the plates and assessing the incorporated ^3H thymidine (1205
10 Betaplate liquid scintillation counter, LKB Wallac) indicated in counts per minute (CPM).

Alternatively, supernatants from the cultures were harvested after 24 hours of incubation with antigen. IL-2 dependent HT-2 cells (5000 cells/well) were added to the culture supernatants and incubated for 16 hours, and the amount of ^3H thymidine
15 incorporated by the cells was determined as in the previous paragraph.

PDC Induced DTH Response in the H-2^k Mouse Model:

Mice (age-matched for each separate experiment) were sensitized by the application of PDC dissolved in DMSO (500 mg/ml) and diluted in acetone (2 mg in
20 100 μl) to the shaved lower abdomen. Mice were routinely challenged 4 days after sensitization by the application of the catechol dissolved as above (30 μg in 10 μl) to both the dorsal and ventral sides of the right ear. The left ear of each mouse was challenged with the solvent (acetone:DMSO) alone as an irritation control. Ear thickness was measured using a spring-loaded micrometer. Results were expressed as
25 the difference in swelling between the right ear and the left ear in units of 10-2 mm. In experiments where other variables were tested, the following controls were included: "primary response" (negative control group) mice were skin-painted with acetone:DMSO and challenged as above; and "secondary response" (positive control group) mice were painted with PDC and challenged as above.

30

Antigen-Specific T Cell Tolerization:

According to the protocol published by G. Shearer and M. Geffer (31) mice were treated with soluble antigen (25-100 μg per mouse) administered wither
intravenously or subcutaneously 10 and 5 days prior to the challenge. The challenge
35 is given subcutaneously (25-100 μg /mouse) in RIBI adjuvant (RIBI Immunochemical, Hamilton, MT).

ANALYSIS:

A. Carrier Modeling and Synthesis:

Carrier Modeling was based on the logic that by knowing the properties of a peptide that are required for binding to a specific MHC class II protein, as well as which residues seem to be generally more important for T cell recognition, it should be possible to design a peptide that will specifically bind the desired MHC class II allele, have little or no immunogenic properties, and provide suitable reactivity for conjugation of a desired hapten at a position that would optimally interact with C cell receptors. Additional constraints of high solubility and aqueous stability were imposed because of the biological necessity of having a soluble compound (carrier: hapten conjugate) for injection into animals or addition to cell culture.

The choice of the I-A^k binding parent peptides HEL 52-61 and IE α 54-66 were made because of their known immunological behavior (31). The requirements for I-A^k binding were determined by measuring the binding of peptides (based on these two parents) that contained single amino acid substitutions at each position of the parent peptide (nominally 19 peptides for each position of the parent sequence), and then elucidating the most probable alignment of the peptide sets (unpublished data), similar to the SAR (structure activity relation) study of the I-A^u system (described in 32). Using the alignments obtained in this fashion, along with the published crystal structure of the human MHC class II allele DRB1*0101 (33), a semi-quantitative model of I-A^k:peptide complexes was formulated.

Using several computer generated models (not shown) of the I-A^k molecule including 2 different peptides (HEL 52-61 (SEQ. ID. NO. 41) and I-E α 54-66 (SEQ. ID. NO. 43)) complexed with the molecule at various angles, it appeared likely that residues in the peptide not closely associated with the I-A^k molecule were necessary requirements for the ability of the peptide to bind the MHC Class II molecule. The binding data for the single amino acid variants of HEL 52-61 (SEQ. ID. NO. 41) and I-E α 54-66 (SEQ. ID. NO. 43) support the latter assumption. Given this information and assumptions, the carrier peptide series A5 (Fig. 5) was designated and synthesized with lysine at the fourth, seventh, or tenth position (peptides A5:4 (SEQ. ID. NO. 47), A5:7 (SEQ. ID. NO. 48), or A5:10 (SEQ. ID. NO. 49) respectively) since these positions are, hypothetically, the most exposed to the TCR (being the least involved with the I-A^k molecule). The remaining residues were determined to be necessary for peptide binding, increasing the solubility of the carrier, and minimizing the exposure of the peptide side chains to putative TCRs.

Fig. 2 lists the peptides made in this study, as well as their solubility in PBS and their affinities for the two H-2^k class II proteins I-A^k and I-E^k. Studies showed that substitution of lysine in the carrier sequence has little effect on the affinity of the peptide for I-A^k (data not shown). Studies also indicated a relative insensitivity of peptide binding to I-A^k when PDC was conjugated to the A5 carrier series (data not shown). The data in Fig. 2 shows that it is possible to maintain or even improve MHC class II binding while dramatically increasing the solubility of the peptide. These results support the determination of which residues are important for MHC class II binding. Fig. 2 illustrates that the I-E^k molecule, in general, binds peptides with a much higher affinity (lower IC₅₀ value) as compared to I-A^k. This phenomenon has been well documented in the literature and somewhat complicates comparison of peptide binding to the two MHC class II proteins. However, the important observation is that the peptides designed to specifically bind I-A^k do not bind I-E^k significantly, and vice versa.

B. The Role of the peptide carrier in Class II binding and T cell recognition

In order to dissect the molecular specificity of T cell recognition of PDC, murine T cell lines specific for PDC were established.

Restriction analysis, utilizing spleen cells from different mouse strains as APC's, revealed that the recognition of A5:7:PDC (SEQ. ID. NO. 48) conjugate is restricted by I-A^k (data not shown). In addition, the T cell line was shown, by FACS analysis, to be CD4⁺ and CD8⁻ (data not shown).

A T cell line established from lymph nodes of C3H/HeN mice immunized with A5:7:PDC (SEQ. ID. NO. 48) show specificity (data not shown). Two discrete peptide carriers with PDC conjugated at the 7th position-A5:7:PDC (SEQ. ID. NO. 48) and I-E α 54-66, K60:PDC (SEQ. ID. NO. 45) activated the T cell line as indicated by ³H-thymidine uptake. In contrast, PDC conjugation at different positions on the carrier-A5:4:PDC (SEQ. ID. NO. 47), A5:10:PDC (SEQ. ID. NO. 49), or the carriers alone gave virtually no response (data not shown). Possible toxicity of A5:4:PDC (SEQ. ID. NO. 47) and A5:10:PDC (SEQ. ID. NO. 49) conjugates or mitogenicity of A5:5:PDC (FEDQKSLENIARD (SEQ. ID. NO. 61)) were ruled out by testing viability of T cells and using naive lymph nodes or an irrelevant, I-A^k restricted T cell line, respectively. These results indicate that conjugation position is crucial for T cell recognition of the hapten. Control peptide:hapten conjugates, consisting of HLA-DR (DR2:7 (SEQ. ID. NO. 6)) of I-E^k (E5:7 (SEQ. ID. NO. 51)) binding peptides with PDC anchored to the 7th position, did not elicit a proliferative

response. These results confirm the importance of MHC binding property of the carrier that is a imperative for PDC-specific, I-A^k restricted T cell recognition.

Syngeneic spleen cells, conjugated with PDC or urushiol, but not TNP, also stimulated this T cell line (data not shown), demonstrating that T cells from
5 A5:7:PDC (SEQ. ID. NO. 48) immunized mice respond to PDC, but not to TNP, when it is conjugated to a variety of carriers. This suggests that these T cells recognize a common determinant in the multiple carrier:hapten conjugates. The results of these experiments indicate that the hapten-specific T cell line, generated using a single carrier coupled to PDC, recognize the hapten in a carrier independent
10 manner.

C. T cells induced by PDC skin-painting can be activated in a hapten-specific fashion by a rationally designed peptide:PDC conjugate

The hapten urushiol binds to epidermal cells, both keratinocytes and
15 Langerhans cells. Langerhans cells conjugated with urushiol then migrate through lymphatics to lymph nodes, where they present antigen (haptenated peptides) to T cells and thereby induce allergic contact sensitivity (twenty-five) to determine whether a hapten specific T cell response, to the carrier:PCD conjugate (A5:7:PCD (SEQ. ID. NO. 48)) can be recalled from draining LN of mice sensitized with PDC
20 following the skin painting. The following studies were conducted.

T cells from draining LN of PDC skin-painted mice were stimulated by A5:7:PDC (SEQ. ID. NO. 48) (data not shown). An unrelated hapten conjugated onto an I-A^k binding carrier (HEL 52-61, K56:TNP (SEQ. ID. NO. 41)), as well as carrier peptides alone, failed to activate these T cells, indicating that PDC sensitization elicits
25 a hapten-specific T cell response. Recognition of A5:7:PDC (SEQ. ID. NO. 48) conjugate by LN T cells from PDC skin painted mice supports the hypothesis that T cell recognition of the hapten can be independent of carrier sequence.

Data showed that T cells primed by PDC skin painting respond to A5:7:PDC but not A5:4:PDC (SEQ. ID. NO. 47) nor A5:10:PDC (SEQ. ID. NO. 49) (data not
30 shown), suggesting that T cell recognition of the hapten is restricted to a specific (7th) position on the carrier.

The lack of response to PDC linked at positions other than the seventh could be due to inferior immunogenicity, low precursor frequency or both. Attempts to generate PDC specific T cell lines by immunization with the A5:4:PDC (SEQ. ID.
35 NO. 47) or A5:10:PDC (SEQ. ID. NO. 49) were unsuccessful, while immunization with the compound A5:7:PDC (SEQ. ID. NO. 48) generated potent, PDC-specific T

cells. Therefore, the seventh position of the carrier peptide appears to be the most favored site for hapten recognition by T cells

D. T cells in PDC sensitized mice fail to recognize PDC in the context of an I-E^k binding peptide

C3H/HeN mice express both I-A^k and I-E^k class II MHC molecules. In order to determine whether a hapten-specific, I-E^k restricted T cell response in mice sensitized with PDC can be obtained, a similar experiment as described in section C was conducted, employing I-E^k binding peptide conjugated to PDC at positions 4th, 7th or 10th.

T cells from draining LN of C3H/HeN mice sensitized with PDC responded to A5:7:PDC (SEQ. ID. NO. 48) but not E5:4:PDC (SEQ. ID. NO. 50), E5:7:PDC (SEQ. ID. NO. 51) or E5:10:PDC (SEQ. ID. NO. 52) (data not shown). Immunization with the above conjugates did not yield a PDC specific T cell. These results suggest that the PDC specific T cell repertoire, triggered by hapten conjugation of cutaneous proteins from PDC skin-painting, is predominantly composed of I-A^k restricted T cells.

E. Antigen-specific, down-regulation of DTH induced by PDC can be achieved by treatment with a single rationally designed peptide-conjugate.

To determine whether a class II binding peptide conjugated to PDC can be used to down regulate a PDC-induced DTH response, C3H/HeN and B10.A(4R) mice were injected with PDC conjugated to different carrier peptides prior to PDC were challenged. Figures 3a and 3b represent two separate experiments. Treatment with A5:7:PDC (SEQ. ID. NO. 48) reduced the DTH response down to background level, indicating that pre-treatment with A5:7:PDC (SEQ. ID. NO. 48) results in downregulation of the effector T cells mediating the DTH response. Treatment with the same carrier peptide coupled to PDC at a different TCR contact residue failed to do so. Additionally, the relatively insoluble carrier:PDC conjugate (I-E α 54-66, K60:PDC (SEQ. ID. NO. 45)) with the hapten conjugated at the preferred TCR contact position, failed to decrease the DTH response in B10.A(4R) mice. This failure to induce downregulation of the DTH response may have been due to the insolubility of the compound.

F. Treatment with peptide:PDC conjugate abrogates the hapten specific T cell proliferative response, and correlates with diminished IL-2 secretion.

To further dissect the downregulation of DTH by peptide:PDC conjugate, the invitro response of cells from LN of pre-injected mice were tested. C3H/HeN and B10.A(4R) mice were treated with either A5:7:PDC (SEQ. ID. NO. 48) ("tolerized") or PBS ("control"), followed by immunization with the above conjugate in the presence of adjuvant (RIBI). T cells from draining LN were tested with a panel of peptide:hapten compounds. Immunized mice demonstrated a vigorous hapten-specific T cell response to A5:7:PDC (SEQ. ID. NO. 48) (Fig. 4a and b). Again, hapten-specific T cells exhibited a narrowly defined specificity that did not cross react with the other conjugates containing PDC at a different TCR contact residue. A carrier specific response was not detected even following deliberate immunization, indicating that we used an nonimmunogenic peptide carrier, ot achieve predominantly a hapten-specific T cell response. T cells from the "tolerized" group failed to proliferate following a challenge with the A5:7:PDC (SEQ. ID. NO. 48) peptide:hapten conjugate (Fig. 4c and d). T cell responses to allogeneic cells or Con-A were similar in the tested groups (data not shown). The above result suggests that treatment with a class II binding peptide coupled to PDC at a discrete position can induce T cell unresponsiveness to the hapten-peptide conjugate.

Failure to proliferate was correlated with diminished IL-2 secretion (data not shown). Exogenous IL-2 added to LN T cells from mice tolerized with A5:7:PDC (SEQ. ID. NO. 48) restored anti-PDC reactivity in a dose dependent fashion (2 and 5 stimulation index at a concentration of 2.5 and 5 U/ml recombinant mouse IL-2, respectively) (data not shown). In spite of the increase of T cell proliferative response in the untreated group, an insignificant change of the stimulation index was observed (10, 10 and 11 stimulation index at a concentration of 0, 2.5 and 5 U/ml recombinant mouse IL-2, respectively). The above result suggests that the unresponsiveness to PDC, induced by treatment with A5:7:PDC (SEQ. ID. NO. 48) conjugate, can be reversed by the additin of exogeneous IL-2, a mechanism consistent with T cell non-responsiveness.

Mitogenic as well as allogeneic responses (mixed lymphocyte reaction - MLR) in the tolerized group were similar to the control group (data not shown) indicating that the state of induced unresponsiveness is confined to the peptide:PDC conjugate alone and is not associated with general immunosuppression.

Similar results were obtained when mice received a challenge of PDC-skin painting (data not shown); T cell responses to A5:7:PDC (SEQ. ID. NO. 48) following PDC skin painting (generating diverse species of hapten conjugated proteins) were down-regulated by pre-treatment (tolerization) with A5:7:PDC (SEQ.

ID. NO. 48), indicating that the tolerance persist even after challenge with varied hapten conjugates.

G. Desensitization: Complete Abrogation of PDC-specific T cell response in A5:7:PDC primed C3H mice

Treatment of PDC-sensitized (primed) mice with a single class II binding peptide:PDC conjugate (A5:7:PDC (SEQ. ID. NO. 48)) was shown to attenuate completely the hapten specific T cell response.

A desensitization experiment was conducted according to the following protocol. C3H mice were primed by injection with 25 µg of A5:7:PDC (SEQ. ID. NO. 48) conjugate in the presence of Ribi (an adjuvant). One month later the mice received 4 weekly injections of A5:7:PDC (SEQ. ID. NO. 48) conjugate (25 µg/injection in PBS, subcutaneously). Two weeks post treatment, the mice were challenged with 25 µg of A5:7:PDC (SEQ. ID. NO. 48) conjugate in the presence of Ribi. Lymph node T cells from the A5:7:PDC (SEQ. ID. NO. 48) desensitized group failed to respond to the haptenic compounds (Fig 6a). Lymph node T cells from primed mice treated with PBS or with the carrier peptide (A5:7 (SEQ. ID. NO. 48)) alone exhibited a vigorous PDC specific response (Figs 6b and Fig. 6c, respectively). Responses to allogenic antigens (MLR) or to Con-A mitogen were intact for all groups.

The efficacy of the treatment in primed mice (desensitization experiment, Fig. 6a) was similar to, if not better than the treatment in naive mice (tolerization experiment, Fig. 6d). Similar tolerization experiments are described above in Example 2F. For comparison, Fig. 6e shows the vigorous response of T cells from unprimed and intolerized mice challenged with A5:7:PDC (SEQ. ID. NO. 48) in the presence of adjuvant.

H. Cross tolerization of PDC specific response using a distinct carrier coupled to PDC at position 7.

In order to demonstrate that the down regulation of the hapten specific T cell response is indeed carrier independent and hapten-specific, a distinct, I-Ak binding carrier, A6:7 (having the amino acid sequence YDDNGAKQNAAER (SEQ. ID. NO. 62)) was coupled to PDC at the 7th position and was used for cross tolerization. C3H mice were treated with A5:7:PDC (SEQ. ID. NO. 48) or A6:7:PDC (SEQ. ID. NO. 62) peptide using the same protocol (2 s.q. injections) and challenged with A5:7:PDC (SEQ. ID. NO. 48). Treatment with A6:7:PDC (SEQ. ID. NO. 62) conjugated was shown to induce unresponsiveness to A5:7:PDC (SEQ. ID. NO. 48) challenge (data

not shown), indicating that the tolerization is specific to the hapten and is independent of the carrier peptide.

5 **Example 3: Haptenated peptides which bind human MHC molecules and stimulate hapten specific T cell responses**

PROCEDURES:

Design /Selection of Carrier Peptides for peptide:PDC conjugation

10 A set of peptides was designed to bind a broad range of HLA DR alleles. The peptides were tested for binding to HLA class II (using representative DR alleles) and their solubility was assessed (see, earlier Examples). In addition, two natural peptides, complement C3 precursor 753-756 and Protein kinase TTK818-830 were selected based on a database criteria using multiple criteria including the following:
15 protein must be a human protein, must be a unique sequence, must have a solubility coefficient that is less than 0 etc. All of the peptides designed or chosen for this study are listed in Fig. 1. One peptide, DR005 (SEQ. ID. NO. 11) was selected and conjugated with PDC at the 7th position as described in Examples 1 and 2. This peptide:PDC conjugate triggered hapted-specific T cell response in DR4Dw4
20 transgenic animals (data not shown) and therefore was put to use in the human T cell proliferation assay system described below.

Initiation of Urushiol-Specific T Cell Cultures

25 Blood samples (~75ml) received from 73 individuals with poison-ivy/oak hypersensitivity, following contact with to poison oak 5-90 days earlier. T cell cultures were initiated from T cells (5×10^4 /well in 96 well Costar plate) separated from the blood following centrifugation on a Ficoll gradient and stimulated with urushiol haptenated autologous cells at a concentration of 25 μ M in AIM-V medium without serum. The urushiol used herein was obtained from the FDA. Urushiol
30 haptenated autologous cells were prepared as follows: Briefly, 2×10^6 cells separated from the blood, resuspended in 200 μ l of PBS and incubated with 10mM of the hapten (urushiol) for 30 minutes at room temperature. The cells are washed three times with PBS, resuspended in 200 μ l of AIM-V and sonicated for 30 seconds using tip sonicator. On day 7 the T cell cultures are fed with fresh AIM-V medium containing
35 10U/ml of rH IL-2 and split or transferred to 24 well Costar plates when necessary. Each patient was given a patient number and each T cell line is referred to with regard to the patient that was stimulated with one antigen.

T cell cloning:

On day 11-14 of the culture T cells are seeded in 96 well Costar plates at a concentration of 0.3 cells/well in the presence of 2×10^4 JY EBV cells (4000R) and 2×10^5 PBLs (3000R) in AIM-V medium containing 1 μ g/ml of PHA and 20U/ml of rH IL-2.

Fourteen days later the growing clones from the 96 well plates are transferred to 24 well plates and are expanded with using the following mixture suspension: 2×10^5 JY EBV cells (4000R) and 2×10^6 PBLs (3000R) in AIM-V medium containing 1 μ g/ml of PHA and 20 U/ml of rH IL-2.

The specificity of the clones is tested (on day 11-14 to the culture) with urushiol-haptenated cells at concentration ranging from 100 μ M to 3.3 μ M in duplicates using 2 fold dilutions. Positive T cell clones are then further tested with various peptide:PDC conjugates to define their fine specificity.

Proliferation Assay

Responder cells are rested without rH IL-2 for 3-4 days prior to the assay. On day 11-14 of the T cell culture, the T cells are collected washed three times to remove IL-2. The cells are then added at 5×10^4 cell/well to U-shape 96 well plates along with 1×10^5 irradiated (3000R) autologous peripheral blood mononuclear cells. Antigens prepared from urushiol, PDC, PIXT (poison ivy extract) or TNP haptenated autologous cells are tested in various concentration ranging from 100 μ M to 3.3 μ M in duplicates using 2 fold dilutions. Preparation of haptenated cells are made freshly for each experiment according to the above protocol. In some experiments DR005:5 (SEQ. ID. NO. 14) (peptide designed to bind wide range of DR alleles) and DR005:7:PDC (SEQ. ID. NO. 16) conjugate were also tested. The antigens were tested in several concentration ranging from 100 μ M to 0.1 μ M. After 72 hours of culture, 100 μ l of supernatant was harvested for assaying release of the lymphokine interferon gamma (using an ELISA kit from Endogen) while the remainder is pulsed with [3H]Thymidine for the final 18 hours of culture. Cells are harvested onto filtermats for liquid scintillation counting T cell proliferation is monitored by thymidine incorporation using Beta plate reader. Supernatants from microtiter plate assay are incubated in ELISA plates coated with anti gIFN, anti IL-4 and anti IL-10 antibodies (Endogene, cytokines kit). Cultures are scored as positive for proliferation if CPM due to tritiated thymidine uptake is greater than or equal to three-fold the background in the unstimulated controls, net counts are at least 1000 CPM, and the

standard error of the mean is less than the net CPM. Stimulation index (SI) is defined as the CPM divided by the CPM of the control.

ANALYSIS

- 5 Hapten specific T cell response was demonstrated in primary cultures from PBL of individuals with recent exposure to poison oak. Fig 5A-C shows the proliferative response of T cells from representative primary T cell cultures (from patients #486, #471, and #551) to urushiol, PDC, and PIXT (poison ivy extract) sonicate of haptenated cells and to DR005:7:PDC (SEQ. ID. NO. 16) conjugate but
10 not to the carrier alone (DR005:7 (SEQ. ID. NO. 16)). These results confirm that T cells are capable of recognizing the arrier peptide with PDC conjugated to the 7th position, but do not recognize the carrier peptide without the PDC

Other studies:

- 15 Using procedures similar to those described above, 5 poison Oak sensitive individuals were screened for urushiol specificity. Several clones from each individual were then assayed for recognition of PDC on 4 different carriers DR005:7:PDC(SEQ. ID. NO. 16), DR011:7:PDC (SEQ. ID. NO. 30) and TTK:7:PDC (SEQ. ID. NO. 2) carrier peptide from human tyrosine kinase protein) and C03:7:PDC
20 (SEQ. ID. NO. 4) (carrier peptide from the third domain of human complement, all as shown in Fig. 5) as well as recognition to PIXT, PDC, urushiol and TNP haptenated autologous cells. Four out of 5 patients responded to all 4 carrier PDC conjugates whereas all patients responded to 3 out of 4 carrier PDC conjugates (Data not Shown). The T cell proliferative response to the various carrier:PDC conjugates varied from
25 patient to patient but not among clones of the same patients. Data showed that T cell clones responding to various carriers also responded to PIXT, PDC, Urushiol but not to TNP haptenated autologous cells demonstrating that T cell specificity is confined to haptens and hapten:conjugates from urushiol but not to unrelated haptens. The data also suggests that T cell recognition of PDC conjugates was independent of the
30 carrier.

Other Studies:

- The effect of the hapten anchoring position onto the class II binding carrier peptide on T cell activation was also studied. The HLA-DR binding carrier, DR005
35 (SEQ. ID. NO. 11) was coupled with PDC at positions 2, 4, 5, 6, 8, 9, 10, 11, and 12 as described in earlier examples. T cell clones from 2 patients responded to DR005 (SEQ. ID. NO. 11) carrier with PDC anchored to various positions in an

undiscriminating fashion. Furthermore EBV lines expressing different HLA-DR were capable of presenting the DR005:PDC conjugate although with different levels of efficiency (Data not shown) This result appears to suggest that recognition of the hapten is completely carrier independent, which as discussed earlier makes possible a
5 "universal type carrier".

Example 4: CD4/CD8 phenotype of primary T cell cultures following antigenic T cell cultures following antigenic stimulation.

10 **FACS analysis:**

The method described by Vedder and Harlan (1988) *J. Clin. Invest.* 81:676, was used in this analysis. Briefly, cells ($0.5-1 \times 10^6$) are incubated with 20 μ l of anti-CD4 antibodies conjugated with PE, together with 20 μ l of anti CD8 antibodies conjugated with FITC for 30 minutes on ice. The cells are washed and monitored for
15 staining using FACS STAR (Becton Dickinson).

Analysis of Results:

In the manner described above, T cells from PBLs of 25 individuals were stained with anti-CD4 and anti CD8 antibodies prior to antigenic stimulation (Day 0)
20 and following primary (P1) and secondary P2 antigenic stimulation. In 24 out of 25 cases, the CD4/CD8 ratio decreased from an average of 3.0 (Day 0) to 1.1 (P1) and 0.8 (P2). This result suggests the induction of CD8+ cells following stimulation with the hapten.

25 **Example 5: Cytokine profile of Urushiol-specific T cell clone from Poison Oak-sensitive patient**

Cytokine Assay:

Cytokine levels in supernatants (from Example 5) were detected by two site
30 sandwich ELISA as previously described (Cherwinski et al, *J. Exp Med.* 166:1229 (1987); Abrams et al, *J. Immunol.*, 140:131 (1988)). Briefly, supernatants from microtiter plate assays are incubated in ELISA plates coated with anti γ IFN, anti IL-4 and anti IL-10 antibodies (Endogen, cytokines kit) followed by incubation with a
secondary matched antibody (supplied in the kit). The plates are washed and the
35 signal is visualized using calorimetric reaction. A standard curved generated by use of known concentration of the cytokines is employed to convert cytokine levels read as O.D to concentration (pg/ml).

Analysis:

Cytokine profile of urushiol specific T cell clone from poison oak-sensitive patients was shown to be that of the TH1 type, classified as the mediators of DTH responses. Analysis of the supernatants from cultures of T cell clone from patient 470.8 with various antigens revealed that high levels of γ IFN, IL-8 and TNF α were secreted whereas no secretion of IL-4 was detected (data not shown).

Example 6: Haptenated peptides which bind to human MHC molecules

A series of haptenated peptides that bind to human MHC Class II molecules HLA-DR1 and/or HLA-DR4 and which have PDC attached to lysines at known T cell contact residues are synthesized.

Choice of carrier peptide

PDC-substituted peptides are prepared using the sequence of influenza H3 hemagglutinin (HA) amino acid residues 307-319 (SEQ ID NO:53). This is a well-characterized native human Class II MHC-binding peptide. Three-dimensional crystallographic data on the binding of this peptide to DR1 have been published (Brown et al. (1993) *Nature* 364:33-39); the binding affinity of the peptide to DR1 and DR4 is low nanomolar; and T cell receptor contact residues at positions 308, 310, 311, 313, and 316 have been identified from substitution analysis (Krieger et al. (1991) *J. Immunol.* 146:2331-2340) as well as from crystallography. The valine residue at position 310 and the asparagine at position 313 are replaced by lysine in separate syntheses to provide carriers that can be PDC-substituted at those sites.

PDC-coupled peptides

Using the approach employed to produce PDC coupled peptides as described in Example 2, a panel of peptides derived from HA₃₀₇₋₃₁₉ (shown in Table 1 below) are produced and characterized. Each peptide is singly substituted with a PDC.

TABLE 1

Panel of DR1/DR4-binding PDC peptides on a HA₃₀₇₋₃₁₉ backbone

- 5 1. HA₃₀₇₋₃₁₉(control - unsubstituted) = PKYVKQNTLKLAT (SEQ. ID. NO. 53)
2. HA₃₀₇₋₃₁₉K308:PDC = PK(PDC)YVKQNTLKLAT (SEQ. ID. NO. 53)
3. HA₃₀₇₋₃₁₉K310:PDC = PKYK(PDC)KQNTLKLAT (SEQ. ID. NO. 57)
4. HA₃₀₇₋₃₁₉K311:PDC = PKYVK(PDC)PNTLKLAT (SEQ. ID. NO. 58)
- 10 5. HA₃₀₇₋₃₁₉K313:PDC = PKYVKQK(PDC)TLKLAT (SEQ. ID. NO. 59)
6. HA₃₀₇₋₃₁₉K316:PDC = PKYVKQNTLK(PDC)LAT (SEQ. ID. NO. 53)

Binding inhibition assays are performed, as described in Example 2 for the
 15 TNP-coupled peptides, to verify the binding of each haptenated HA peptide to
 isolated DR4 molecules.

Establishing and testing T cell lines derived from DR1 and DR4 donors of known
 urushiol hypersensitivity to define *in vitro* antigenicity of each peptide

T cell clones are established from several DR1 and DR4 donors who have
 20 experienced rashes from natural contact with *Rhus* species. To assure an adequate
 source of peripheral blood as a source of T cells and APC, appropriate tissue-typed
 volunteers donate up to 1 unit of blood for these studies. The published procedure of
 Kalish et al. is followed in essential detail to establish T cell lines (Kalish and
 Johnson (1990) *J. Immunol.* 145:3703-3713). Heparinized peripheral venous blood is
 25 separated into peripheral mononuclear cells on Ficoll-Hypaque and T cells are
 isolated by erythrocyte rosetting. The resulting peripheral blood T cells are plated in
 U-bottom 96 well plates, using the inner 60 wells, along with 2×10^5 /well irradiated
 (4000R) peripheral blood mononuclear cells and antigen in the form of the PDC-
 coupled carriers used at concentrations beginning at 50 μ g/ml. The antigen
 30 concentration is a key factor that is systematically varied at initiation of replicate
 cultures for optimal stimulation. Carriers are hen eggwhite lysozyme (HEL-PDC),
 ovalbumin (OVA-PDC) and keyhole-limpet hemocyanin (KLH-PDC). Several
 carriers are tested since all may not be processed and presented equally well by each
 individual's peripheral blood monocytes, and because T cells specific for some
 35 carriers may be stimulated in certain individuals. However, after the initial expansion
 and pre-screening, lines are assayed for recognition of PDC-coupled versus
 uncoupled peptides not related to the initial carriers. T cells added per well are $3 \times$

10², 1 × 10³, 3 × 10³, 9 × 10³ and 3 × 10⁴ in 60 replicates each. Culturing is performed in Gibco AIM V medium, 1% human AB serum, supplemented with glutamine and antibiotics. After 6 days, recombinant IL-2 (40 U/ml) and IL-4 (19 U/ml) are added to all cultures. Growing cultures are assayed after an additional
5 week for response to the initiating antigens. A sample of 75 µl from each culture is transferred to a 96-well round-bottom plate and washed. The remaining culture is fed with medium/IL-2/IL-4. Each sample is washed and split to 6 wells (three duplicates) containing freshly-thawed autologous, irradiated PBL and either PDC-antigen, carrier only or medium only. After 48 hrs, assay wells are pulsed with [³H]thymidine and
10 counted. Positives are selected for expansion according to the following criteria: a) stimulation index (CPM experimental/CPM control) ≥ 3.0, b) Δ CPM ≥ 1000, c) standard error < Δ CPM. Cultures positive on the initiating antigen are expanded to 48 well plates in the presence of IL-2/IL-4 for 6-8 days and tested in duplicate for proliferation in response to each of the synthetic peptides.

15

Proliferation assay of T cell lines using PDC-peptides:

Responder cells are rested without lymphokines for 3-4 days prior to assays. Cells are washed three times to remove IL-2, resuspended in fresh AIM V medium, 1% AB serum, and added at 5 × 10⁴ cells/well to flat-bottomed 96 well plates along
20 with 1 × 10⁵ irradiated autologous peripheral blood monuclear cells. Each 48-well culture is split into 14 wells (final volume 200 microliters) for testing duplicate cultures of each of the control and PDC-peptides at 1-10 micromolar in the panel as well as a medium-only control. After 24 hours of culture, 100 µl of supernatant is
25 harvested for assaying release of the lymphokine interferon-gamma (antibodies for immunoassays provided by Dr. Giorgio Trinchieri, Wistar Institute) while the remainder are pulsed with [³H]thymidine for the final 18 hours of culture. Cells are harvested onto filtermats for liquid scintillation counting.

Analysis of results:

30 Cultures are scored as positive for proliferation if CPM due to tritiated thymidine uptake is greater than or equal to three-fold the background in the unstimulated controls, net counts are at least 1000 CPM, and the standard error of the mean is less than the net CPM. For interferon gamma assays, backgrounds are
usually below the level of detection (<1 U/ml) while stimulated cultures produce 80-
35 100 U/ml (Kalish and Johnson (1990) *J. Immunol.* 145:3706-3713). Since the peptides are DR-binding, Class II MHC-restricted T cells rather than Class I-restricted responses are measured.

These studies provide information on the ability of PDC-coupled peptides to be recognized by human T cells. Principally, data on the T cell antigenicity of each peptide is obtained and conditions for assays are established. A determination of a significant response to each peptide is made, and the frequency of PDC-peptide reactive T cells from different donors is approximated. Peptides that are widely recognized in urushiol-responsive patients can then be tested for efficacy in a delayed-type hypersensitivity model in transgenic mice expressing human DR4 Class II MHC molecules in a manner described in Example 3.

In these mice, typical hapten-specific hypersensitivity reactions can be mediated (e.g., as described in Example 3) by hapten-specific T cells which recognize the hapten bound to DR4 molecules. Thus, these mice can be used to examine the ability of PDC-coupled peptides which bind to DR4 to induce urushiol-specific T cell nonresponsiveness and desensitize urushiol-sensitized subjects.

Example 7: Coupling of penicillamine to a MHC-binding peptide

Penicillamine (3-mercapto-D-valine) was coupled to a cysteine residue within a synthetic MHC Class II-binding peptide having the amino acid sequence:

Ala-Ala-Tyr-Lys-Ala-Ala-Cys-Ala-Ala-Ala-Ala-Ala (SEQ. ID. NO. 60)

1 2 3 4 5 6 7 8 9 10 11 12 13

Iodine was used as an oxidant to permit disulfide bonding between the cysteine sulfhydryl and the mercapto group of D-penicillamine. To the peptide in acetic acid was added 10 equivalents of D-penicillamine, followed by addition of iodine/acetic acid until the solution turned slightly yellow, eventually becoming colorless. Then an additional 10 equivalents of iodine/acetic acid was added until a stable color developed. Mixture was stirred 1 hr, then rotary evaporated to remove acetic acid/iodine, and the peptide purified by HPLC. Mass spectroscopy confirmed the coupling.

The penicillamine-substituted peptide is tested for recognition by penicillamine-reactive T cell isolated from blood donated from arthritis patients taken off penicillamine after exhibiting a sensitivity. Demonstration of specific T cell recognition could lead to a desensitization regimen in line with the principles discussed herein.

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
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- 10 (A) NAME: IMMULOGIC PHARMACEUTICAL CORPORATION
(B) STREET: 610 Lincoln Street
(C) CITY: Waltham
(D) STATE: MA
(E) COUNTRY: USA
15 (F) POSTAL CODE (ZIP): 02154
(G) TELEPHONE: (617) 466-6000
(H) TELEFAX: (617) 466-6040
- 20 (ii) TITLE OF INVENTION: HAPTENATED PEPTIDES AND USES THEREOF
- (iii) NUMBER OF SEQUENCES: 62
- 25 (iv) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/222,206
(B) FILING DATE: April 1, 1994
- 35 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Vanstone, Darlene A.
(B) REGISTRATION NUMBER: 35,279
(C) REFERENCE/DOCKET NUMBER: 079.2PCT
- 40 (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: (617) 466-6000
(B) TELEFAX: (617) 466-6040
- 45 (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- 50 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 55 (v) FRAGMENT TYPE: internal
- 60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
- Glu His Tyr Ser Gly Gly Glu Ser His Asn Ser Ser Ser

1

5

10

(2) INFORMATION FOR SEQ ID NO: 2:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

20 Glu His Tyr Ser Gly Gly Lys Ser His Asn Ser Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

40 Glu Asp Ile Ile Ala Glu Glu Asn Ile Val Ser Arg Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

60 Glu Asp Ile Ile Ala Glu Lys Asn Ile Val Ser Arg Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

- 65 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
Ala Ala Ile Ala Ser Ala Ala Ser Ala Ala Ala Gln Ala
1 5 10

15 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
Ala Ala Ile Ala Ser Ala Lys Ser Ala Ala Ala Gln Ala
1 5 10

35 (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
Asp Ala Ile Ala Ser Ala Ala Ser Ala Ala Ala Asn Glu
1 5 10

55 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
60 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

65 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
Asp Ala Ile Ala Ser Ala Lys Ser Ala Ala Ala Asn Glu
1 5 10
- 10 (2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
15 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
20
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
Asp Ala Ile Ala Ser Ala Ala Asn Ala Ala Ala Asn Glu
1 5 10
- 30 (2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
35 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
40
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
Asp Ala Ile Ala Ser Ala Lys Asn Ala Ala Ala Asn Glu
1 5 10
- 50 (2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
55 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
60
- 65 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
Asp Ala Ile Ala Ser Ala Ala Gln Ala Ala Ala Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20 Asp Lys Ile Ala Ser Ala Ala Gln Ala Ala Ala Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

- 30 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

40 Asp Ala Ile Lys Ser Ala Ala Gln Ala Ala Ala Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 14:

- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

- 50 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

60 Asp Ala Ile Ala Lys Ala Ala Gln Ala Ala Ala Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 15:

- 65 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 5 (v) FRAGMENT TYPE: internal
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
- Asp Ala Ile Ala Ser Lys Ala Gln Ala Ala Ala Asn Glu
1 5 10
- 15 (2) INFORMATION FOR SEQ ID NO: 16:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
- 20 (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 25 (v) FRAGMENT TYPE: internal
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
- Asp Ala Ile Ala Ser Ala Lys Gln Ala Ala Ala Asn Glu
1 5 10
- 35 (2) INFORMATION FOR SEQ ID NO: 17:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
- 40 (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 45 (v) FRAGMENT TYPE: internal
- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
- Asp Ala Ile Ala Ser Ala Ala Lys Ala Ala Ala Asn Glu
1 5 10
- 55 (2) INFORMATION FOR SEQ ID NO: 18:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
- 60 (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 65 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

5 Asp Ala Ile Ala Ser Ala Ala Gln Lys Ala Ala Asn Glu
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

25 Asp Ala Ile Ala Ser Ala Ala Gln Ala Lys Ala Asn Glu
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

45 Asp Ala Ile Ala Ser Ala Ala Gln Ala Ala Lys Asn Glu
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

65 Asp Ala Ile Ala Ser Ala Ala Gln Ala Ala Ala Lys Glu
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Asp Ala Ile Ala Ser Ala Ala Ser Ala Ala Ala Arg Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Asp Ala Ile Ala Ser Ala Lys Ser Ala Ala Ala Arg Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Ala Tyr Ala Ser Ala Ala Ser Ala Ala Ala Asn Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

- 5 (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
Asp Ala Tyr Ala Ser Ala Lys Ser Ala Ala Ala Asn Glu
1 5 10
- 15 (2) INFORMATION FOR SEQ ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
20 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
Asp Ala Ile Ala Ser Ala Ala Ser Ala Ala Ala Asn Asp
1 5 10
- 30 (2) INFORMATION FOR SEQ ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
40 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:
Asn Ala Ile Ala Ser Ala Ala Ser Ala Ala Ala Asn Asp
1 5 10
- 50 (2) INFORMATION FOR SEQ ID NO: 28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
60 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

5 Ser Ala Ile Ala Ala Asn Ala Ser Ala Ala Ala Asn Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

25 Ser Ala Tyr Ala Ala Asn Ala Ser Ala Ala Ala Asn Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 30:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

45 Ser Ala Tyr Ala Ala Asn Lys Ser Ala Ala Ala Asn Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: peptide

 (v) FRAGMENT TYPE: internal

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

65 Ala Gly Tyr Arg Ser Asn Tyr Thr Tyr Tyr Ala Tyr Ala
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 32:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 10 (v) FRAGMENT TYPE: internal
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:
Ala Gly Tyr Arg Ser Asn Tyr Thr Ala Gln Ala Gln Ala
1 5 10
- 20 (2) INFORMATION FOR SEQ ID NO: 33:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
25 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 30 (v) FRAGMENT TYPE: internal
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:
Gly Gly Tyr Ala Gly Glu Ala Gly Pro Ala Ala Gly Gly
1 5 10
- 40 (2) INFORMATION FOR SEQ ID NO: 34:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
45 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 50 (v) FRAGMENT TYPE: internal
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:
Thr Leu Ser Ala Ala Ala Ala Asn Leu
1 5
- 60 (2) INFORMATION FOR SEQ ID NO: 35:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
65 (C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

10 Thr Leu Ser Lys Ala Ala Ala Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 36:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

30 Thr Leu Ser Ala Lys Ala Ala Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 37:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

50 Thr Leu Ser Ala Ala Ala Lys Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 38:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Ala Ala Tyr Lys Ala Ala Lys Ala Ala Ala Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Asp Tyr Gly Ile Leu Gln Ile Asn Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Asp Lys Gly Ile Leu Gln Ile Asn Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Asp Tyr Gly Ile Lys Gln Ile Asn Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:
Asp Tyr Gly Ile Leu Gln Ile Lys Ser Arg
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 43:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:
Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 44:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:
Phe Glu Ala Lys Gly Ala Leu Ala Asn Ile Ala Val Asp
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 45:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

10 Phe Glu Ala Gln Gly Ala Lys Ala Asn Ile Ala Val Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 46:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

30 Phe Glu Ala Gln Gly Ala Leu Ala Asn Lys Ala Val Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 47:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

50 Phe Glu Asp Lys Gly Ser Leu Glu Asn Ile Ala Arg Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 48:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

65

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Phe Glu Asp Gln Gly Ser Lys Glu Asn Ile Ala Arg Asp
1 5 10

5 (2) INFORMATION FOR SEQ ID NO: 49:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
10 (C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (v) FRAGMENT TYPE: internal

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Phe Glu Asp Gln Gly Ser Leu Glu Asn Lys Ala Arg Asp
1 5 10

25 (2) INFORMATION FOR SEQ ID NO: 50:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
30 (C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (v) FRAGMENT TYPE: internal

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Ala Ala Ile Lys Ala Ala Ala Ala Ala Arg Ala Ala
1 5 10

45 (2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
50 (C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (v) FRAGMENT TYPE: internal

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Ala Ala Ile Ala Ala Ala Lys Ala Ala Arg Ala Ala
1 5 10

65 (2) INFORMATION FOR SEQ ID NO: 52:

- (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

15 Ala Ala Ile Ala Ala Ala Ala Ala Lys Arg Ala Ala
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 53:

20

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

35 Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 54:

40

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

55 Ala Ala Tyr Ala Ala Ala Ala Ala Lys Ala Ala Ala
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 55:

60

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: peptide

- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:
5 (A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/note= "Wherein Xaa is alanine or lysine or cysteine"
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:
Ala Ala Tyr Xaa Ala Ala Xaa Ala Ala Lys Ala Ala Ala
1 5 10
- 15 (2) INFORMATION FOR SEQ ID NO: 56:
(i) SEQUENCE CHARACTERISTICS:
20 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
25 (v) FRAGMENT TYPE: internal
(ix) FEATURE:
30 (A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/note= "Wherein Xaa is alanine or lysine or cysteine"
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:
Ala Ala Ile Xaa Ala Ala Xaa Ser Ala Xaa Ala Ala Ala
1 5 10
- 40 (2) INFORMATION FOR SEQ ID NO: 57:
(i) SEQUENCE CHARACTERISTICS:
45 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
50 (v) FRAGMENT TYPE: internal
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:
Pro Lys Tyr Lys Lys Gln Asn Thr Leu Lys Leu Ala Thr
1 5 10
- 60 (2) INFORMATION FOR SEQ ID NO: 58:
(i) SEQUENCE CHARACTERISTICS:
65 (A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 5
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:
- 10 Pro Lys Tyr Val Lys Pro Asn Thr Leu Lys Leu Ala Thr
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 59:
- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
20 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 25
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:
- 30 Pro Lys Tyr Val Lys Gln Lys Thr Leu Lys Leu Ala Thr
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 60:
- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
40 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal
- 45
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:
- 50 Ala Ala Tyr Lys Ala Ala Cys Ala Ala Ala Ala Ala Ala
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 61:
- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
60 (C) STRANDEDNESS:
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
65 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

5 Phe Glu Asp Gln Lys Ser Leu Glu Asn Ile Ala Arg Asp
 1 5 10

10 (2) INFORMATION FOR SEQ ID NO: 62:

 (i) SEQUENCE CHARACTERISTICS:

 (A) LENGTH: 13 amino acids
15 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

20 (v) FRAGMENT TYPE: internal

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

 Tyr Asp Asp Asn Gly Ala Lys Gln Asn Ala Ala Glu Arg
 1 5 10

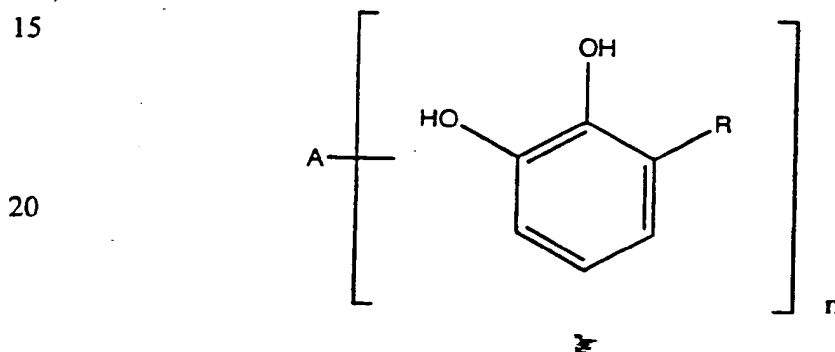
CLAIMS

1. A haptenated peptide having the structure



wherein A represents a peptide of from 7 to 30 amino acid residues capable of binding to a Class II MHC molecule, B represents a hapten molecule, n represents 1, 2 or 3, and — represents a covalent bond linking each hapten molecule (B) to an amino acid residue of the peptide (A).

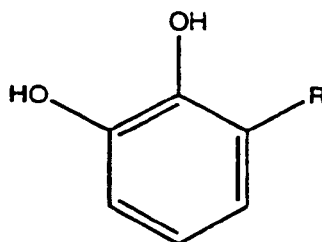
2. The peptide of claim 1 which has the structure



25 wherein R is a saturated or unsaturated hydrocarbon radical of from 0 to 20 carbon atoms; A represents a peptide of from 7 to 30 amino acid residues capable of binding to a Class II MHC molecule; n represents 1, 2 or 3; and — represents a covalent bond.

3. The peptide of claim 2 wherein R is an unbranched hydrocarbon chain.
- 30 4. The peptide of claim 2 wherein R is of 8 to 15 carbon atoms.
5. The peptide of claim 2 wherein R is of 8 to 10 carbon atoms.
- 35 6. The peptide of claim 2 wherein n is 1.
7. The peptide of claim 2 wherein A is a peptide of 9 to 15 amino acids, of which at least one internal (non-terminal) amino acid has a nucleophilic side chain.

8. The peptide of claim 2 wherein A is a peptide of 9 to 13 amino acids, of which at least one internal (non-terminal) amino acid has a nucleophilic side chain.
9. The peptide of claim 8 which has at least one internal lysine residue.
- 5 10. A human Class II MHC-binding compound including the structure
(Xaa)_n-Zaa-Xaa-Xaa-Zaa-Xaa-Xaa-Zaa-(Xaa)_m
wherein Xaa and Zaa represent amino acids, and at least one Zaa is covalently bound to a hapten molecule; and n and m represent integers from 1 to 3, with n + m
10 equalling 2 to 6.
11. The compound of claim 10 wherein at least one Zaa is selected from the group of lysine, cysteine, tyrosine, arginine, serine, and histidine.
- 15 12. The compound of claim 11 wherein the hapten molecule has the structure;

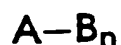


25

wherein R is a C₀-20 hydrocarbon radical which may be branched, unbranched or cyclic, and may be saturated or unsaturated.

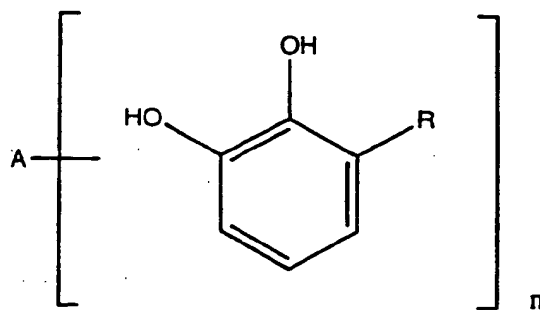
- 30 13. The compound of claim 12 wherein R is *n*-alkyl or *n*-alkenyl of 8 to 15 carbons.
14. The compound of claim 13 wherein R is *n*-alkyl or *n*-alkenyl of 8 to 10 carbon atoms.
- 35 15. The compound of claim 12 wherein R is pentadecyl.

16. A human Class II MHC-binding compound having the structure



wherein A is a human Class II-binding peptide selected from the group consisting of:
 TTK (SEQ. ID. NO. 1), TTK:7 (SEQ. ID. NO. 2), C03 (SEQ. ID. NO. 3), C03:7
 (SEQ. ID. NO. 4), DR002:0 (SEQ. ID. NO. 5), DR002:7 (SEQ. ID. NO. 6), DR003:0
 (SEQ. ID. NO. 7), DR003:7 (SEQ. ID. NO. 8), DR004:0 (SEQ. ID. NO. 9), DR004:7
 (SEQ. ID. NO. 10), DR005:0 (SEQ. ID. NO. 11), DR005:10 (SEQ. ID. NO. 19),
 DR005:11 (SEQ. ID. NO. 20), DR005:12 (SEQ. ID. NO. 21), DR005:2 (SEQ. ID.
 NO. 12), DR005:4 (SEQ. ID. NO. 13), DR005:5 (SEQ. ID. NO. 14), DR005:6 (SEQ.
 ID. NO. 15), DR 005:7 (SEQ. ID. NO. 16), DR005:8 (SEQ. ID. NO. 17), DR005:9
 (SEQ. ID. NO. 18), DR006:0 (SEQ. ID. NO. 22), 006:7 (SEQ. ID. NO. 23), 007:0
 (SEQ. ID. NO. 24), 007:7 (SEQ. ID. NO. 25), 008:0 (SEQ. ID. NO. 26), DR009:0
 (SEQ. ID. NO. 27), DR010:0 (SEQ. ID. NO. 28), DR011:0 (SEQ. ID. NO. 29),
 DR011:7 (SEQ. ID. NO. 30), DR012:0 (SEQ. ID. NO. 31), DR013:0 (SEQ. ID. NO.
 32), DR014:0 (SEQ. ID. NO. 33), HLA001:0 (SEQ. ID. NO. 34), HLA001:4 (SEQ.
 ID. NO. 35), HLA001:5 (SEQ. ID. NO. 36), HLA001:6 (SEQ. ID. NO. 37) all as
 shown in Fig. 1. Preferred peptides include DR005:7 (SEQ. ID. NO. 16), DR011:0
 (SEQ. ID. NO. 29), DR011:7 (SEQ. ID. NO. 30), TTK:7 (SEQ. ID. NO. 2), and
 CO3:7 (SEQ. ID. NO. 4) all as shown in Fig. 1, B is a hapten molecule; n is an integer
 from 1 to 3; and — represents a covalent bond between each B and the side chain of a
 non-terminal amino acid residue of A.

17. The compound of claim 16 wherein B has the structure



wherein R is a saturated or unsaturated hydrocarbon radical of from 0 to 20 carbon atoms.

18. The compound of claim 17 wherein R is an unbranched hydrocarbon chain of at least 8 carbon atoms and no more than about 10 carbon atoms.
- 5 19. The compound of claim 17 wherein n is 1.
20. The compound of claim 16 wherein B is a urushiol.
21. The compound of claim 16 wherein B is a pentadecyl catechol group.
- 10 22. The compound of claim 16 wherein A is selected from the group consisting of: DR005 (SEQ. ID NO. 11), DR005:7 (SEQ. ID NO. 16), DR011:7 (SEQ. ID NO. 30), TTK:7 (SEQ. ID NO. 2), and CO3:7 (SEQ. ID NO. 4) all as shown in Fig. 1, and B is a urushiol or PDC.
- 15 23. The compound of claim 22 wherein B is covalently bound to A at the amino acid residue in position 4, 7, 9, 10, or 11 of A.
24. The compound of claim 23 wherein B is covalently bound to A at the amino acid residue in position 7 of A.
- 20 25. A human Class II MHC-binding compound having the structure
- $$\text{A}-\text{B}_n$$
- wherein A is a human Class II-binding peptide selected from the group consisting of
- 25 Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (SEQ. ID NO. 53)
Pro-Lys-Tyr-Lys-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr (SEQ. ID NO. 57)
Pro-Lys-Tyr-Val-Lys-Gln-Lys-Thr-Leu-Lys-Leu-Ala-Thr (SEQ. ID NO. 59)
Ala-Ala-Tyr-Ala-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala (SEQ. ID NO. 54)
Ala-Ala-Tyr-Zaa-Ala-Ala-Zaa-Ala-Ala-Lys-Ala-Ala-Ala (SEQ. ID NO. 55)
- 30 Ala-Ala-Ile-Zaa-Ala-Ala-Zaa-Ser-Ala-Xaa-Ala-Ala-Ala (SEQ. ID NO. 56)
wherein Zaa is selected from the group Lys, Cys, Ala, Tyr, His, Asn, Ser, Thr;
B is a hapten molecule; n is an integer from 1 to 3; and — represents a covalent bond between each B and the side chain of a non-terminal amino acid residue of A.
- 35 26. A composition useful for treating contact sensitivity to urushiol comprising at least one haptenated compound of claim 16 and a pharmaceutically acceptable carrier or diluent.

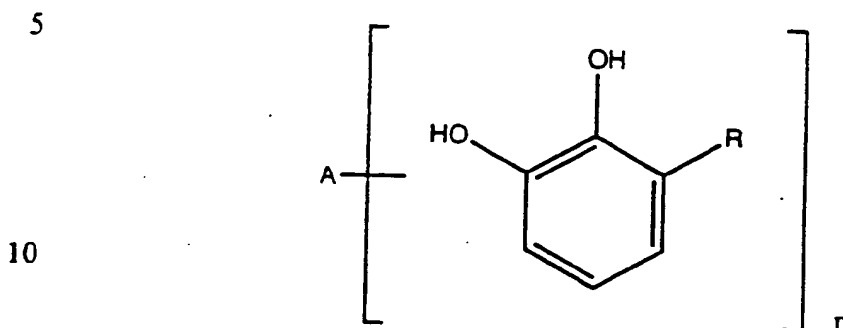
27. A composition useful for treating contact sensitivity to urushiol comprising at least one haptenated compound of claim 22 and a pharmaceutically acceptable carrier of diluent.
- 5 28. A composition useful for treating contact sensitivity to urushiol comprising at least one haptenated compound of claim 24 and a pharmaceutically acceptable carrier or diluent..
- 10 29. A method for desensitizing a mammal against a contact allergen comprising administering to the mammal at least one composition of claim 26.
30. A method for desensitizing a mammal against a contact allergen comprising administering to the mammal simultaneously or sequentially at least two different
- 15 compositions of claim 26.
31. A method for desensitizing a mammal against a contact allergen comprising administering to the mammal at least one composition of claim 24.
- 20 32. A method for desensitizing a mammal against a contact allergen comprising administering to the mammal at least one composition of claim 29.
33. A haptenated compound having the structure

25



- wherein A is selected from the group consisting of: a peptide of from 7 to 30 amino acid residues capable of binding to a Class II MHC molecule, a peptide from 8-11 amino acids long capable of binding to a Class I MHC molecule, and a lipid capable
- 30 of binding a CD1b molecule; B represents a hapten molecule, n represents 1, 2 or 3, and — represents a covalent bond linking each hapten molecule (B) to an amino acid residue of the peptide (A).

34. The compound of claim 33 which has the structure



wherein R is a saturated or unsaturated hydrocarbon radical of from 0 to 20 carbon atoms; n represents 1, 2 or 3; and — represents a covalent bond.

35. The compound of claim 34 wherein A is a peptidomimetic comprising at least one peptide bond alternative selected from the group consisting of: an N-methyl amide bond ($\text{NH-C}_{\alpha 2}[-\text{CO-NCH}_3-]\text{C}_{\alpha 1}$) and a reduced bond analog ($\text{NH-C}_{\alpha 2}[-\text{CH}_2\text{-NH-}]\text{C}_{\alpha 1}$).

36. The compound of claim 35 wherein R is an unbranched hydrocarbon chain of at least 8 carbons in length.

37. The compound of claim 35 wherein A is a peptidomimetic having the structure:



wherein [-] is a normal peptide bond, [*] is an N-Methyl amide bond or a normal peptide bond and [\$] is a reduced bond analog or a normal peptide bond.

38. A composition useful for treating contact sensitivity to urushiol comprising at least one compound of claim 36 and a pharmaceutically acceptable carrier or diluent.

39. A method for desensitizing a mammal against a contact allergen comprising administering to said mammal at least one composition of claim 38.

40. A composition useful for treating contact sensitivity to urushiol comprising at least three different compounds of claim 33 wherein at least one compound comprises a class II binding peptide, wherein at least one compound comprises a class I binding peptide, and wherein at least one compound comprises a CD1b binding lipid; and a pharmaceutically acceptable carrier or diluent.
- 5

10

HUMAN SYSTEM PEPTIDES NAME	SEQUENCE
TTK 818-830 (TTK)	Ac-E H Y S G G E S H N S S S-NH2
TTK 818-830 K824 (TTK:7)	Ac-E H Y S G G K S H N S S S-NH2
C. C3.P 753-765 (C03)	Ac-E D I I A E E N I V S R S-NH2
C.C3.P753-765 K759 (C03:7)	Ac-E D I I A E K N I V S R S-NH2
DR BINDER 002:0	Ac-A A I A S A A S A A A Q A-NH2
DR BINDER 002:7	Ac-A A I A S A K S A A A Q A-NH2
DR BINDER 003:0	Ac-D A I A S A A S A A A N E-NH2
DR BINDER 003:7	Ac-D A I A S A K S A A A N E-NH2
DR BINDER 004:0	Ac-D A I A S A A N A A A N E-NH2
DR BINDER 004:7	Ac-D A I A S A K N A A A N E-NH2
DR BINDER 005:0	Ac-D A I A S A A Q A A A N E-NH2
DR BINDER 005:2	Ac-D K I A S A A Q A A A N E-NH2
DR BINDER 005:4	Ac-D A I K S A A Q A A A N E-NH2
DR BINDER 005:5	Ac-D A I A K A A Q A A A N E-NH2
DR BINDER 005:6	Ac-D A I A S K A Q A A A N E-NH2
DR BINDER 005:7	Ac-D A I A S A K Q A A A N E-NH2
DR BINDER 005:8	Ac-D A I A S A A K A A A N E-NH2
DR BINDER 005:9	Ac-D A I A S A A Q K A A N E-NH2
DR BINDER 005:10	Ac-D A I A S A A Q A K A N E-NH2
DR BINDER 005:11	Ac-D A I A S A A Q A A K N E-NH2
DR BINDER 005:12	Ac-D A I A S A A Q A A A K E-NH2
DR BINDER 006:0	Ac-D A I A S A A S A A A R E-NH2
DR BINDER 006:7	Ac-D A I A S A K S A A A R E-NH2
DR BINDER 007:0	Ac-D A Y A S A A S A A A N E-NH2
DR BINDER 007:7	Ac-D A Y A S A K S A A A N E-NH2
DR BINDER 008	Ac-D A I A S A A S A A A N D-NH2
DR BINDER 009	Ac-N A I A S A A S A A A N D-NH2
DR BINDER 010	Ac-S A I A A N A S A A A N D-NH2
DR BINDER 011	Ac-S A Y A A N A S A A A N D-NH2
DR BINDER 011:7	Ac-S A Y A A N K S A A A N D-NH2
DR BINDER 012	Ac-A G Y R S N Y T Y Y A Y A-NH2
DR BINDER 013	Ac-A G Y R S N Y T A Q A Q A-NH2
DR BINDER 014	Ac-G G Y A G E A G P A A G G-NH2
HLA-A2 BINDER 001:0	Ac-T L S A A A A N L-NH2
HLA-A2 BINDER 001:4	Ac-T L S K A A A N L-NH2
HLA-A2 BINDER 001:5	Ac-T L S A K A A N L-NH2
HLA-A2 BINDER 001:6	Ac-T L S A A A K N L-NH2

Fig. 1

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SUBSTITUTE SHEET (RULE 26)

Fig. 2

PEPTIDE NAME	SEQUENCE	I-Ak IC ₅₀ VALUE μ(M)	I-Ek IC ₅₀ VALUE μ(M)	SOLUBILITY (mg/ml)
HADP7.47	H:-AAYKAAKAAAAA-NH2	4.7	>100	10
HEL 52-61	AC-DYGILQINSR-NH2	2.5	>100	1
HEL 52-61, K53	AC-DKGILQINSR-NH2	3.2	>100	3
HEL 52-61, K53 (TNP)	AC-DK (TNP) GILQINSR-NH2	5.1	>100	0.4
HEL 52-61, K53 (PDC)	AC-DK (PDC) GILQINSR-NH2	NA	NA	NA
HEL 52-61, K56	AC-DYGIKQINSR-NH2	4.8	>100	2
HEL 52-61, K56 (TNP)	AC-DYGIK (TNP) QINSR-NH2	3.8	>100	0.25
HEL 52-61, K56 (PDC)	AC-DYGIK (PDC) QINSR-NH2	NA	NA	NA
HEL 52-61, K59	AC-DYGILQIKSR-NH2	5.8	>100	3
HEL 52-61, K59 (TNP)	AC-DYGILQIK (TNP) SR-NH2	6.5	>100	0.3
HEL 52-61, K59 (PDC)	AC-DYGILQIK (PDC) SR-NH2	NA	NA	NA
I-Eα54-66	H:-FEAQGALANIAVD-OH	5.1	>100	2.5
I-Eα54-66, K57	AC-FEAKGALANIAVD-NH2	4.8	>100	4.5
I-Eα54-66, K60	AC-FEAQAGAKANIAVD-NH2	5.6	>100	6.0
I-Eα54-66, K63	AC-FEAQGALANKAVD-NH2	6.0	>100	2.8
I-Eα54-66, K57 (PDC)	AC-FEAK (PDC) GALANIAVD-NH2	NA	NA	NA
I-Eα54-66, K60 (PDC)	AC-FEAGAK (PDC) ANIAVD-NH2	NA	NA	NA
I-Eα54-66, K63 (PDC)	AC-FEAQGALANK (PDC) AVD-NH2	NA	NA	NA
A5:4	AC-FEDKGSLENIARD-NH2	7.0	>100	>20
A5:4:PDC	AC-FEDK (PDC) GSLENIARD-NH2	3.5	12.0	0.5
A5:7	AC-FEDQGSKEIARD-NH2	5.0	>100	>20
A5:7:PDC	AC-FEDQGSK (PDC) ENIARD-NH2	6.2	>100	0.6
A5:10	AC-FEDQGSLENKARD-NH2	8.0	>100	>20
A5:10:PDC	AC-FEDQGSLENK (PDC) ARD-NH2	7.1	>100	1.2
E5:4	AC-AAIKAAAAAARA-NH2	>100	0.125	>20
E5:4:PDC	AC-AAIK (PDC) AAAAAARA-NH2	>100	0.220	0.7
E5:7	AC-AAIAAAKAAAAA-NH2	>100	0.280	>20
E5:7:PDC	AC-AAIAAAK (PDC) AAAAAA-NH2	>100	0.300	0.5
E5:10	AC-AAIAAAKAAAAA-NH2	>100	0.150	>20
E5:10:PDC	AC-AAIAAAK (PDC) RAA-NH2	>100	0.230	0.8

*NA = NOT APPLICABLE DUE TO INSOLUBILITY OF COMPOUND.

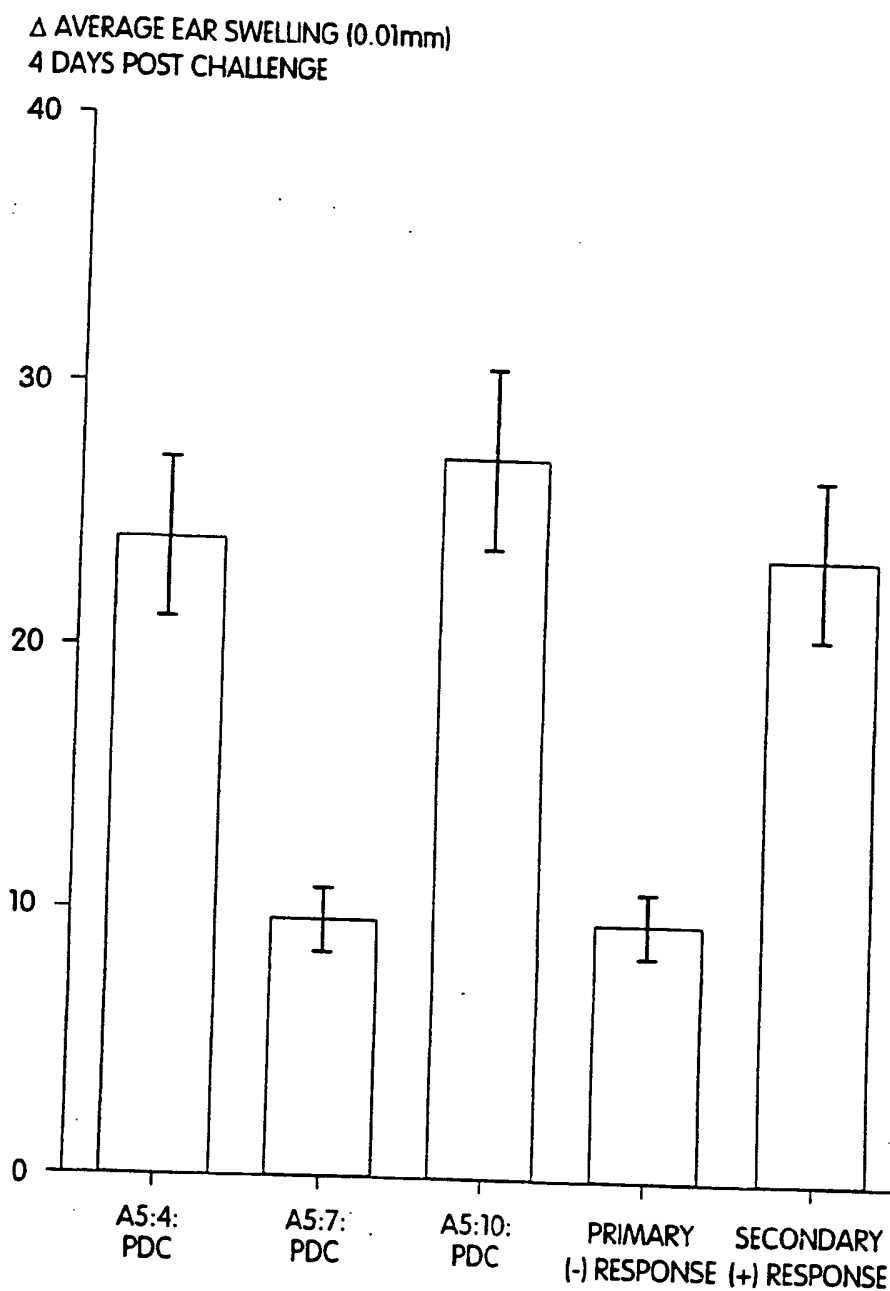


Fig. 3a

Δ AVERAGE EAR SWELLING (0.01mm)
4 DAYS POST CHALLENGE

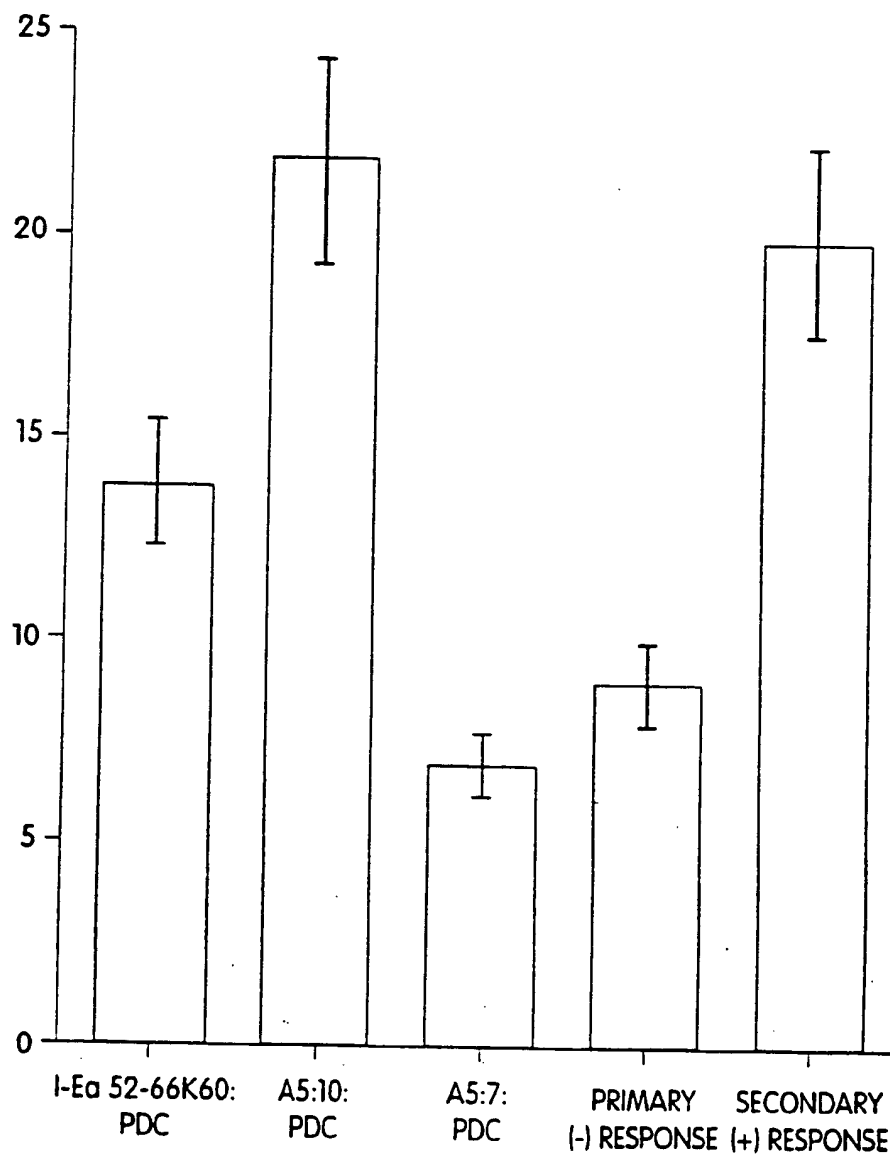


Fig. 3b

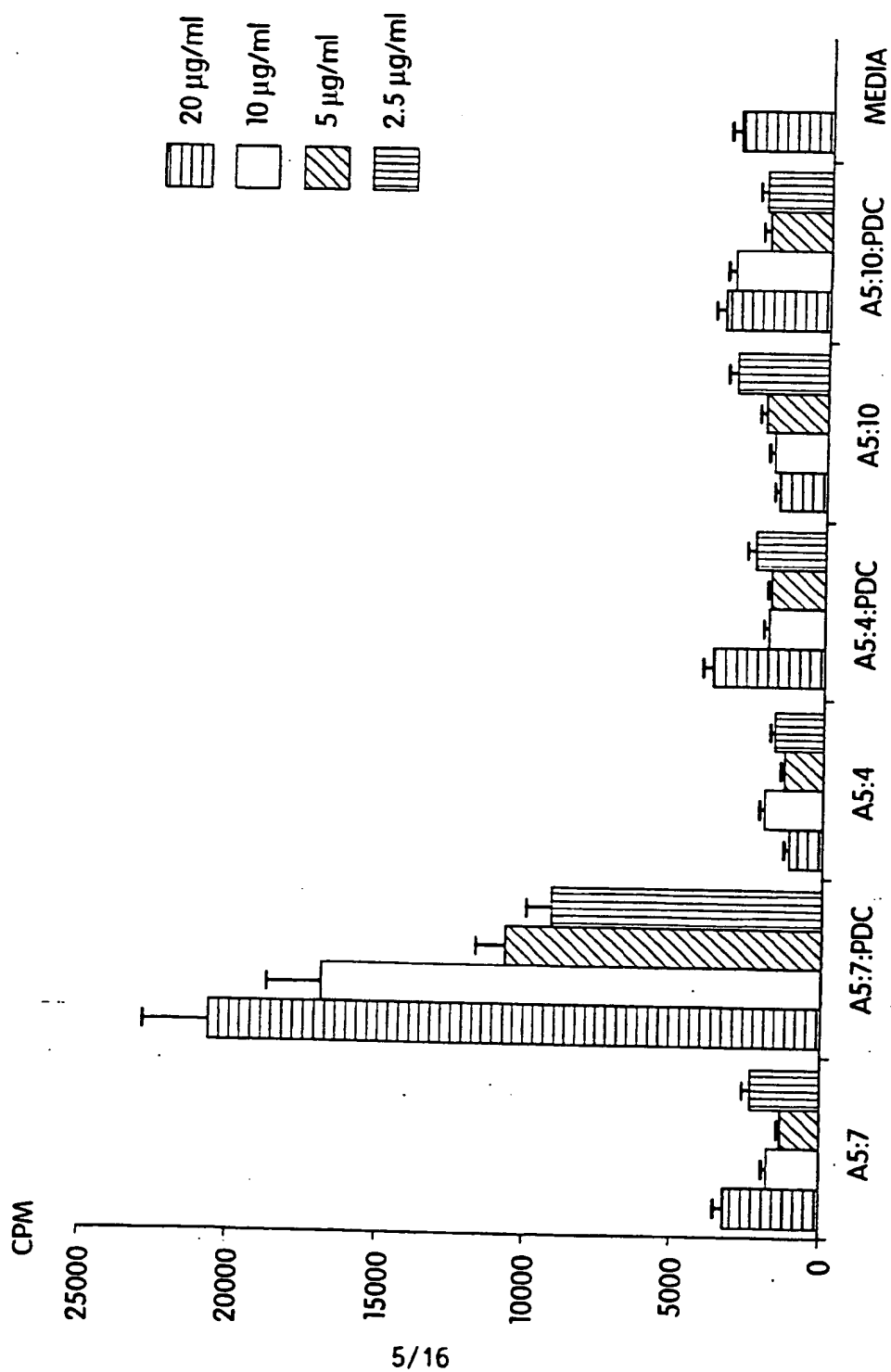
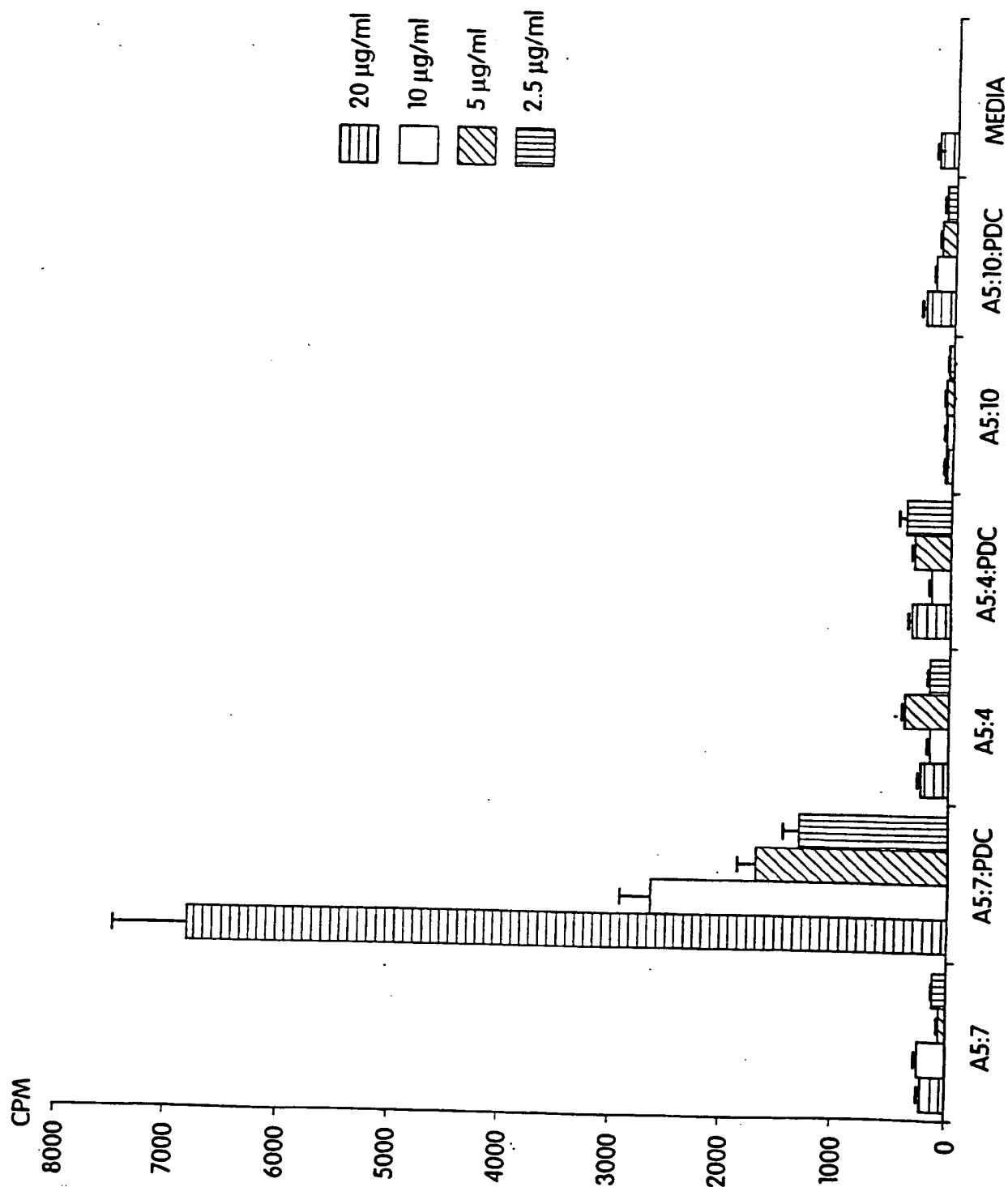


Fig. 4a



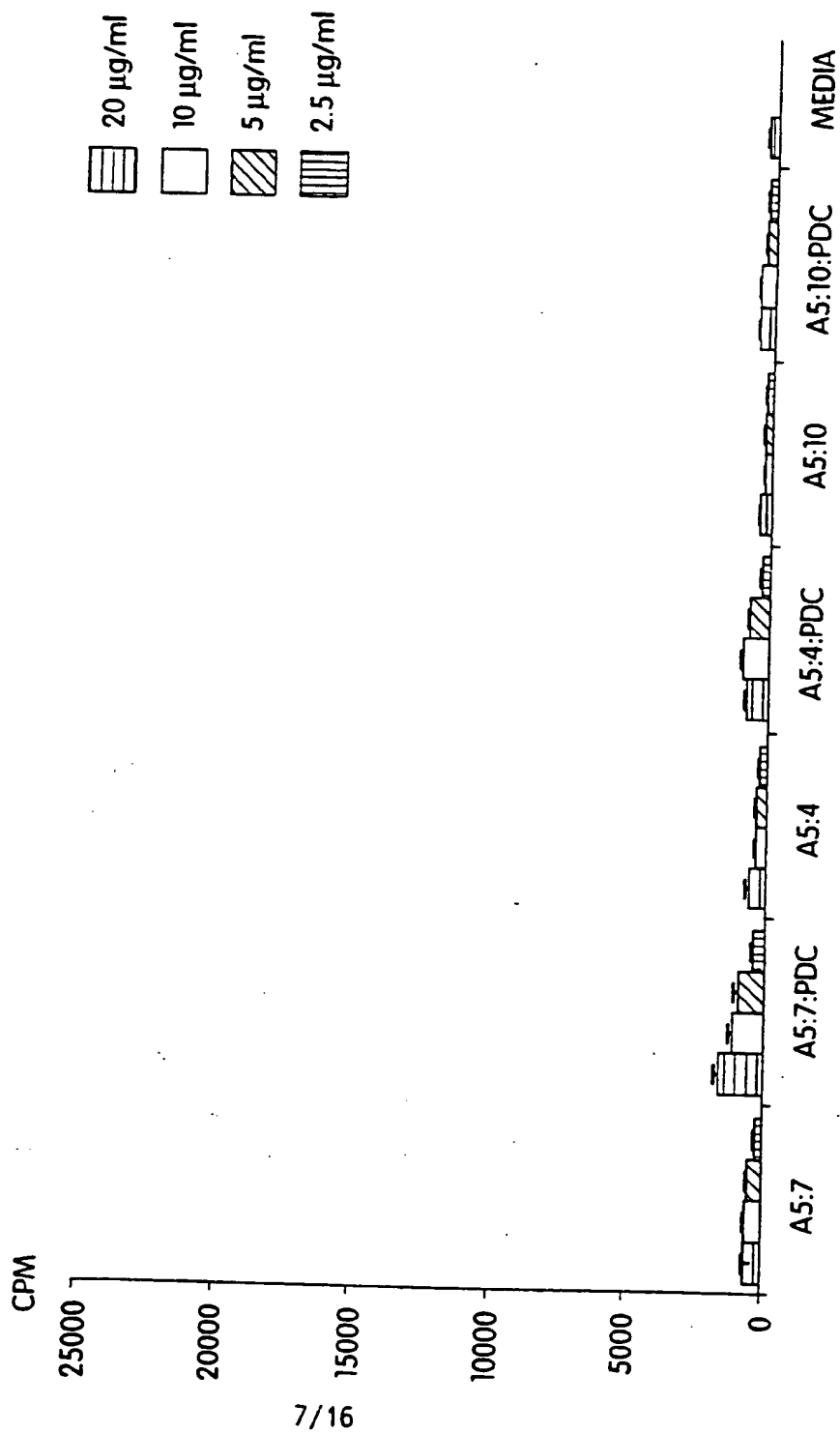


Fig. 4c

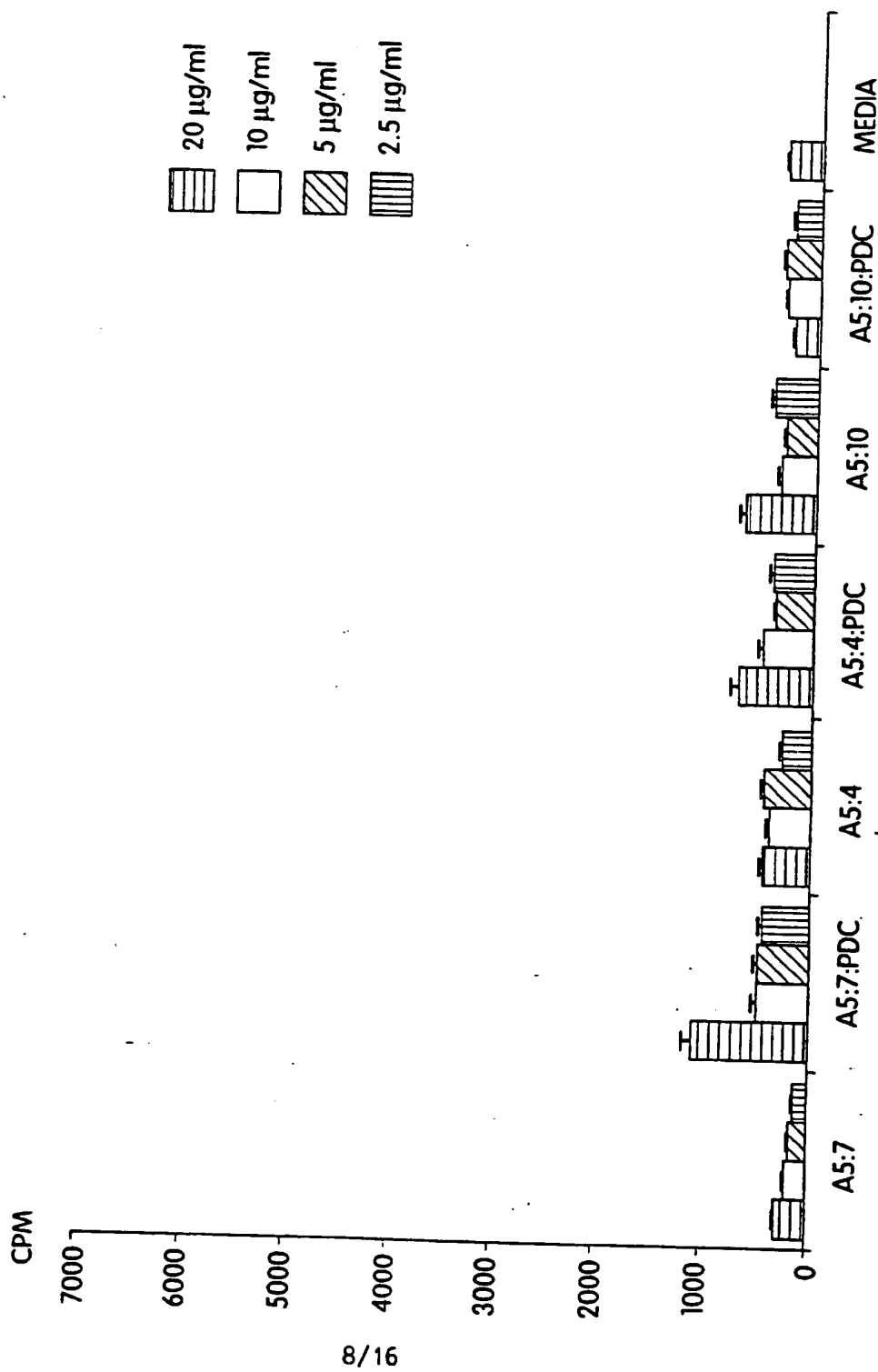


Fig. 4d

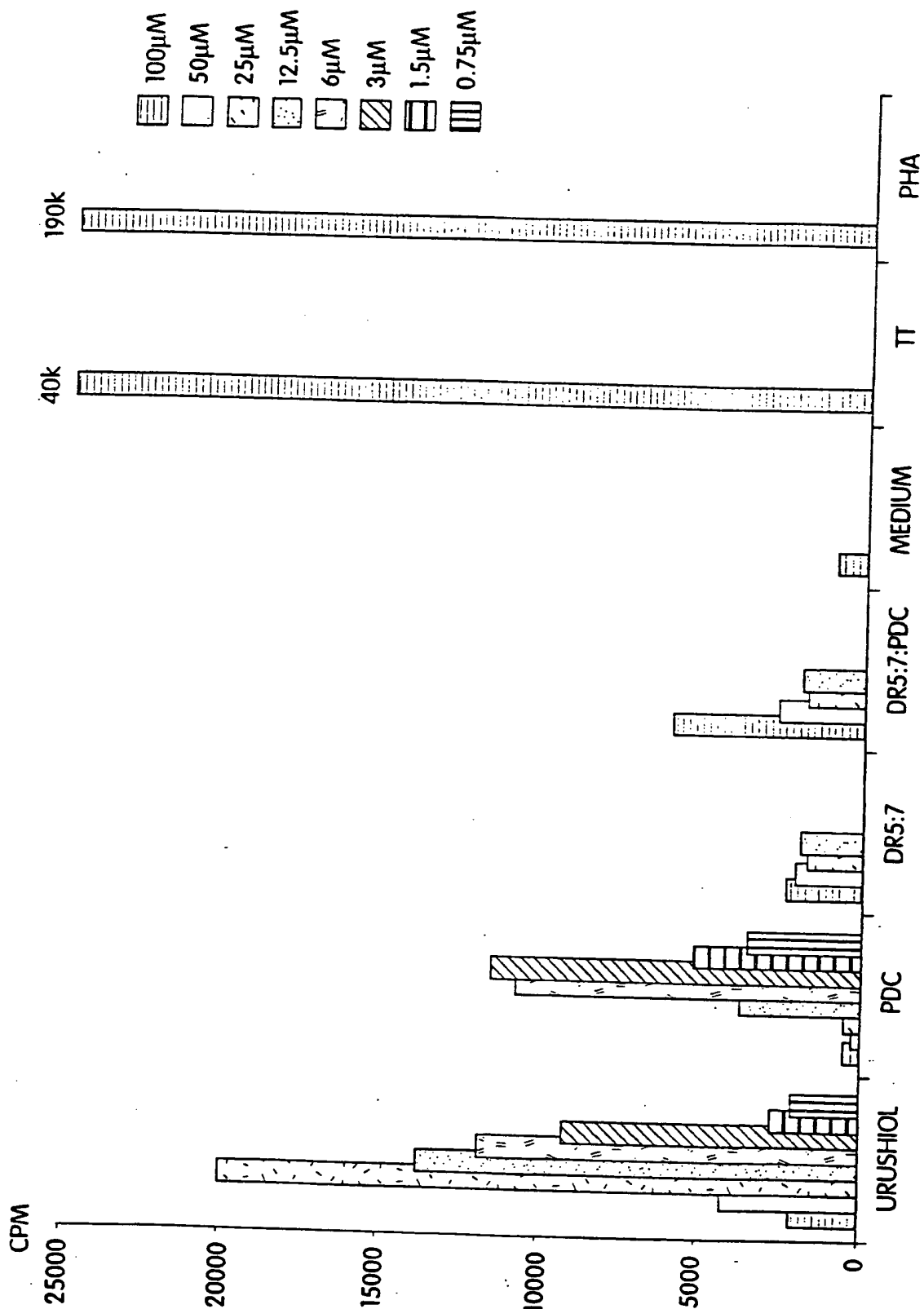


Fig. 5a

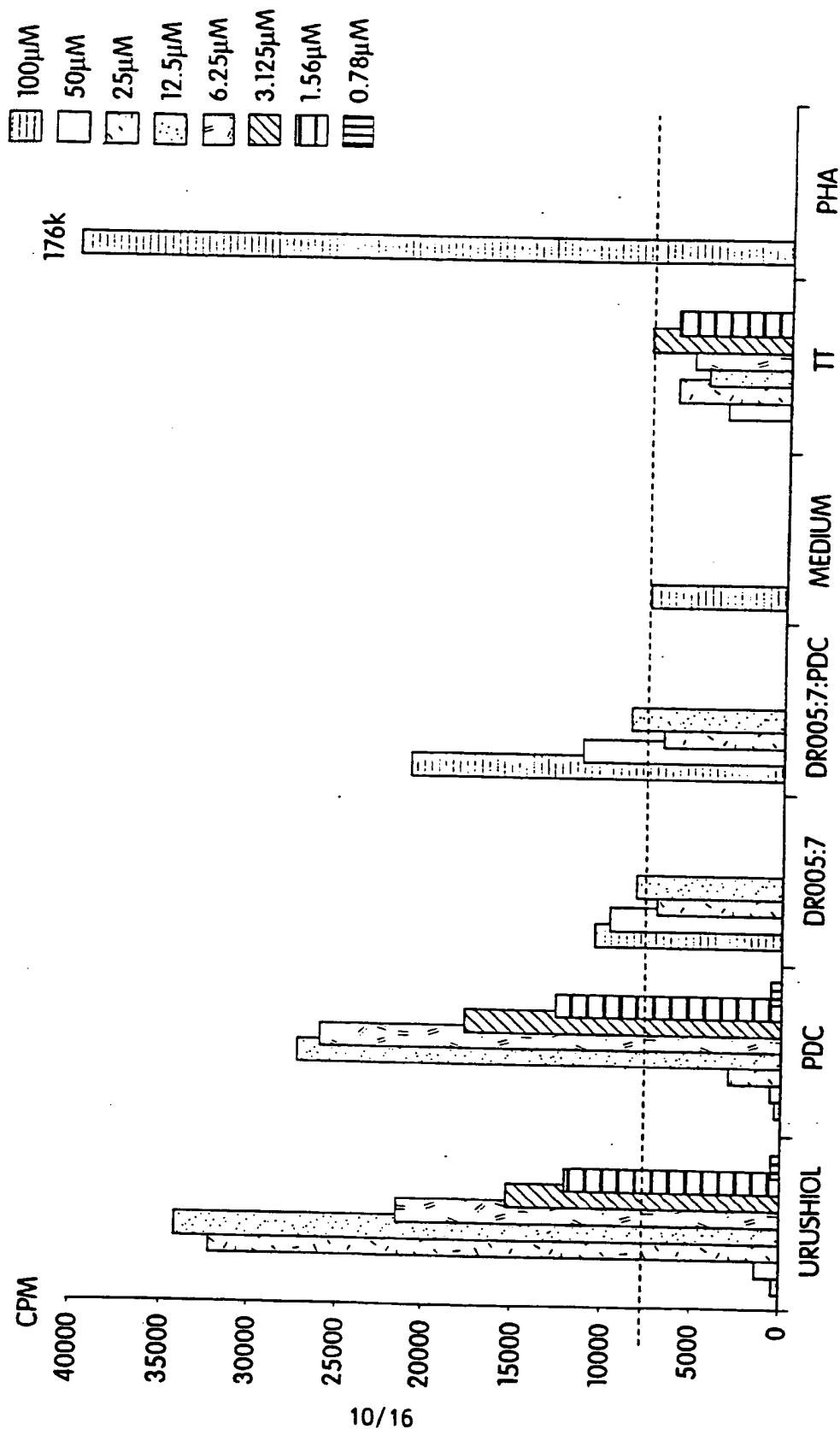


Fig. 5b

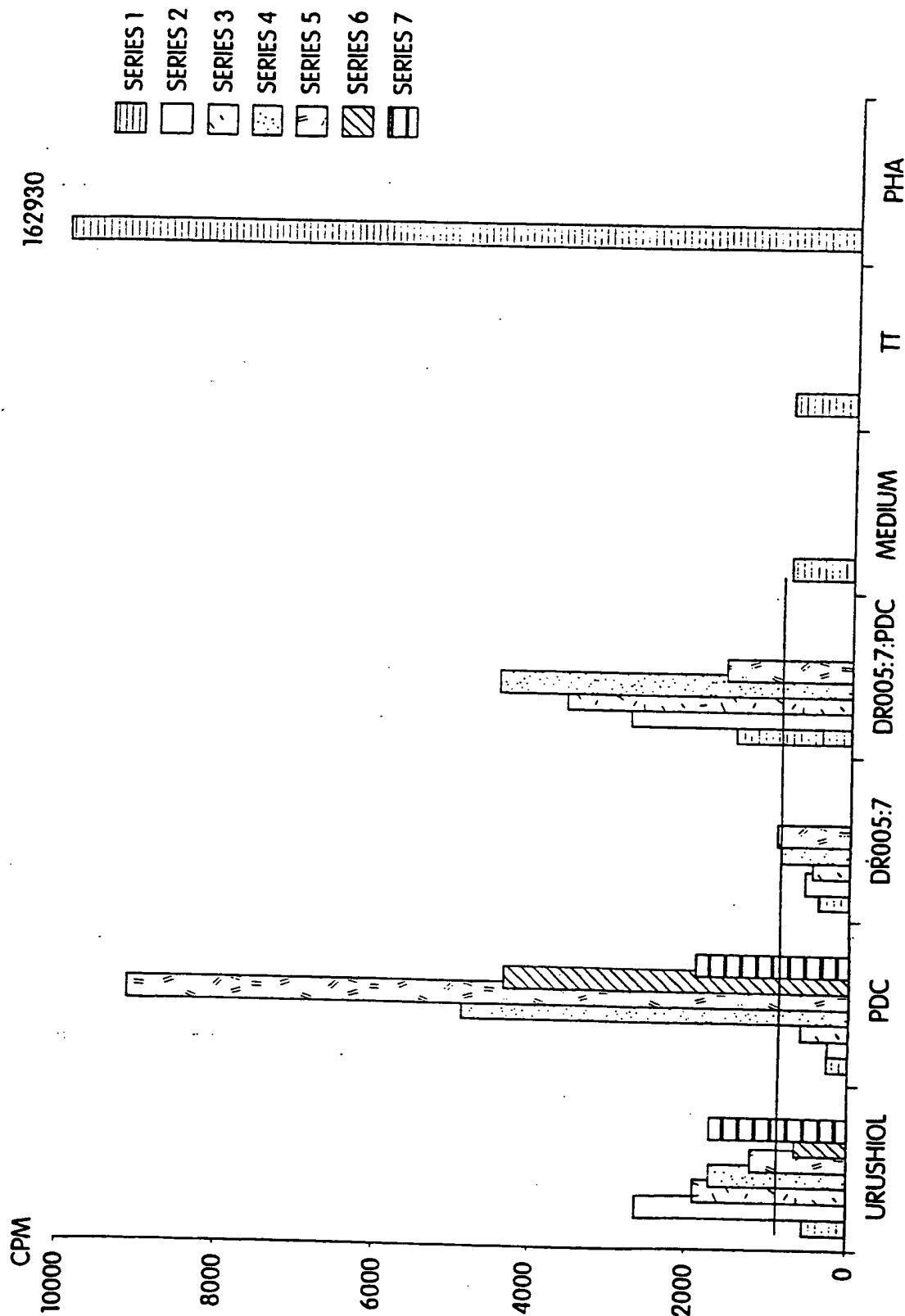


Fig. 5c

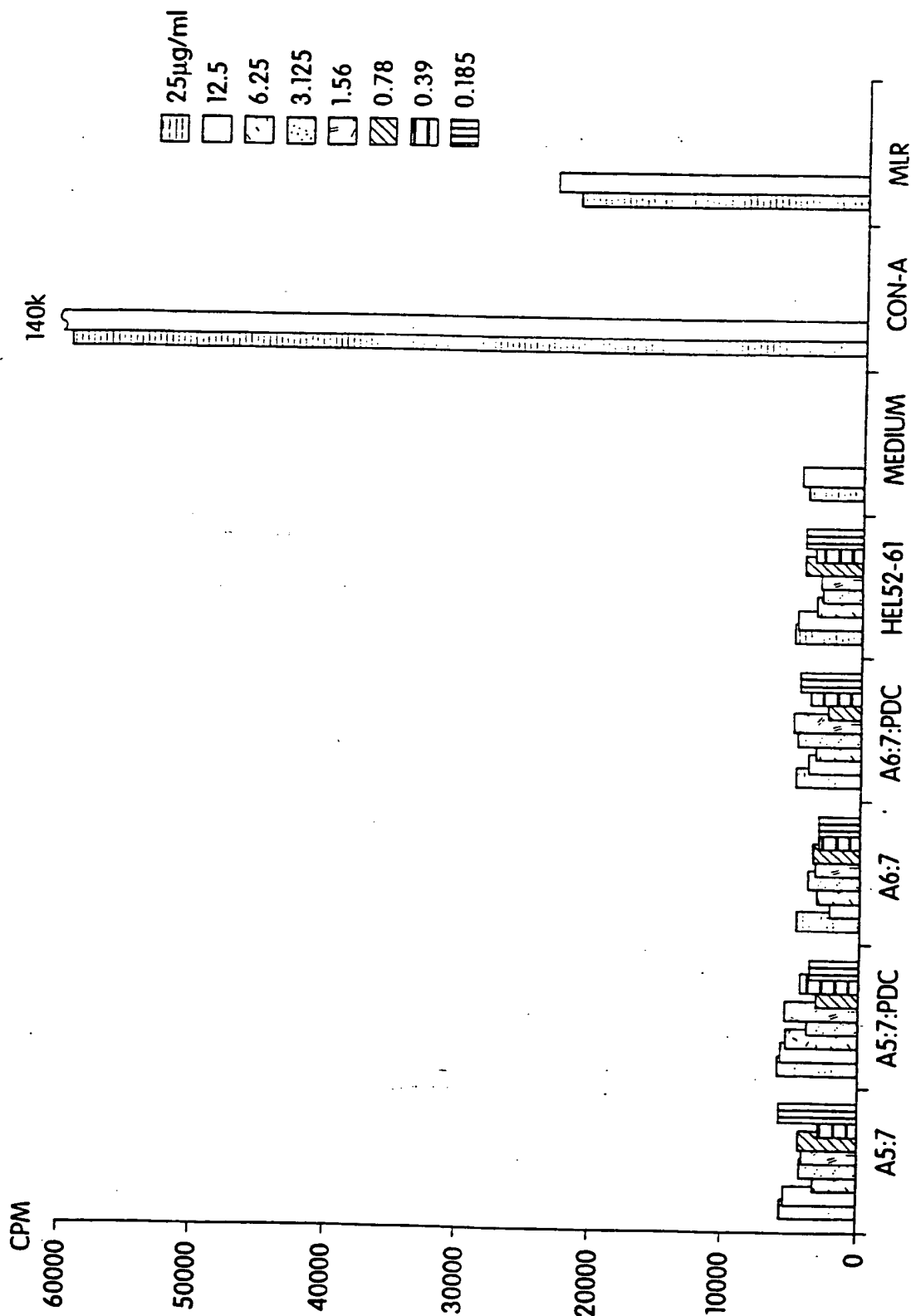


Fig. 6a

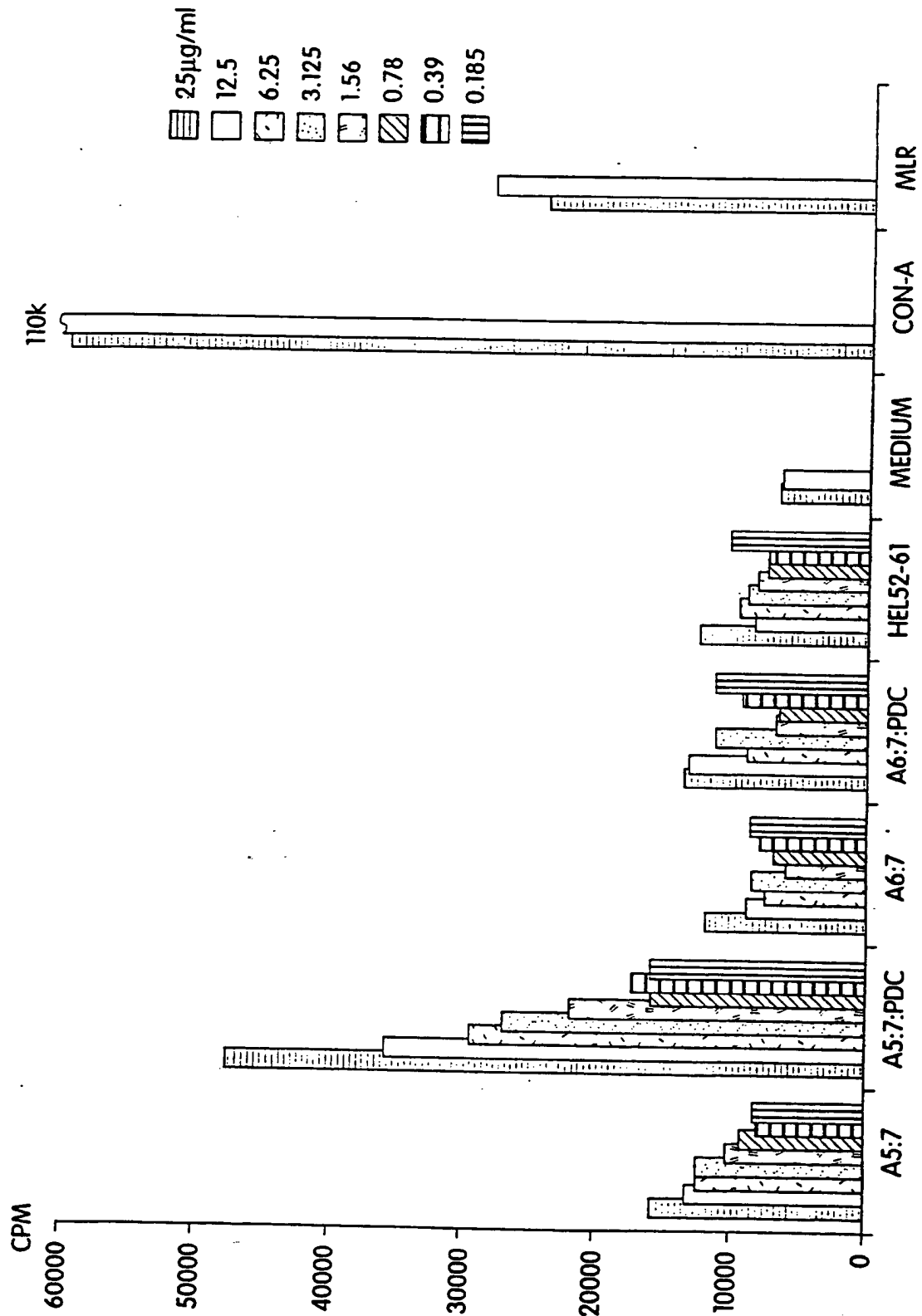


Fig. 6b

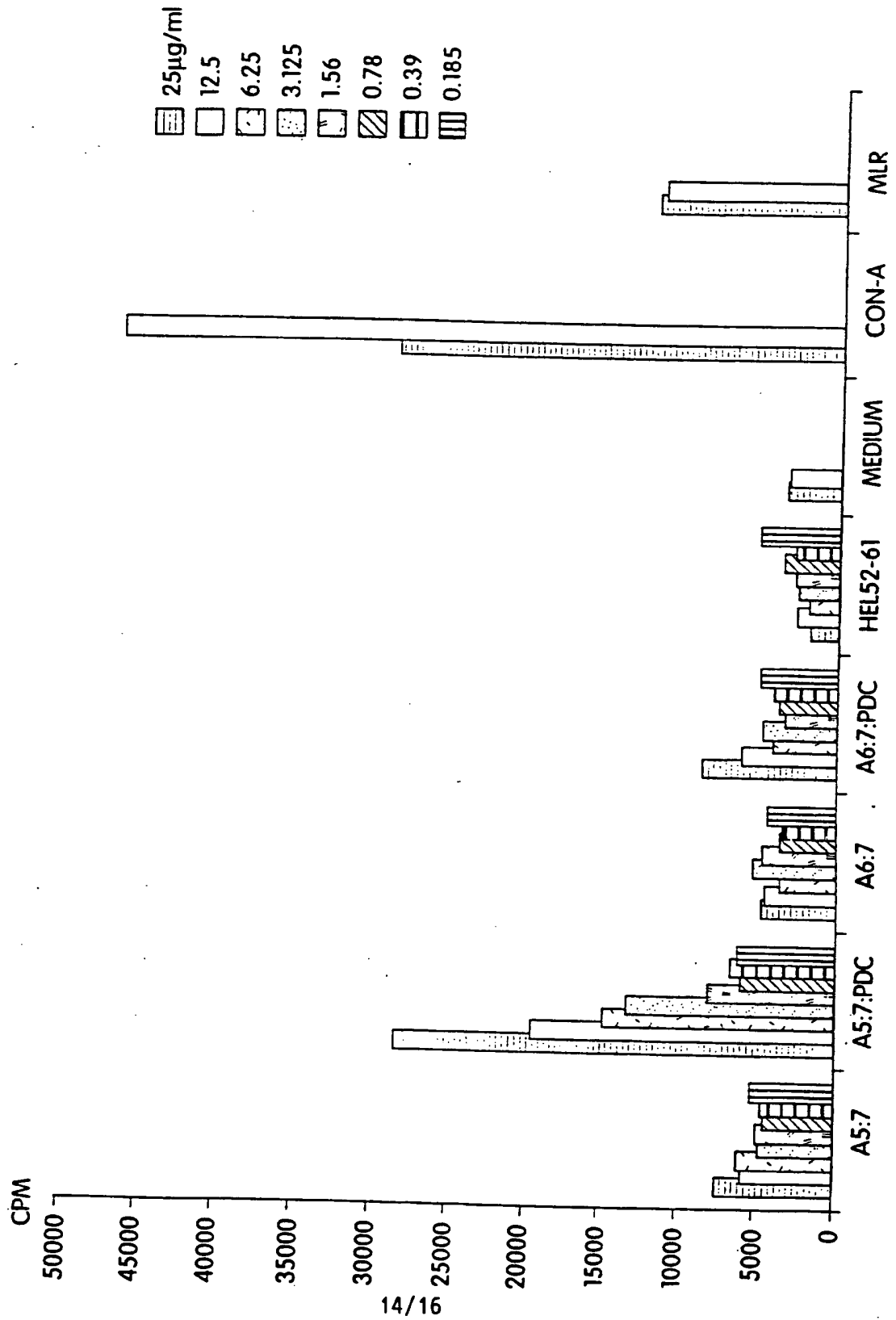


Fig. 6c

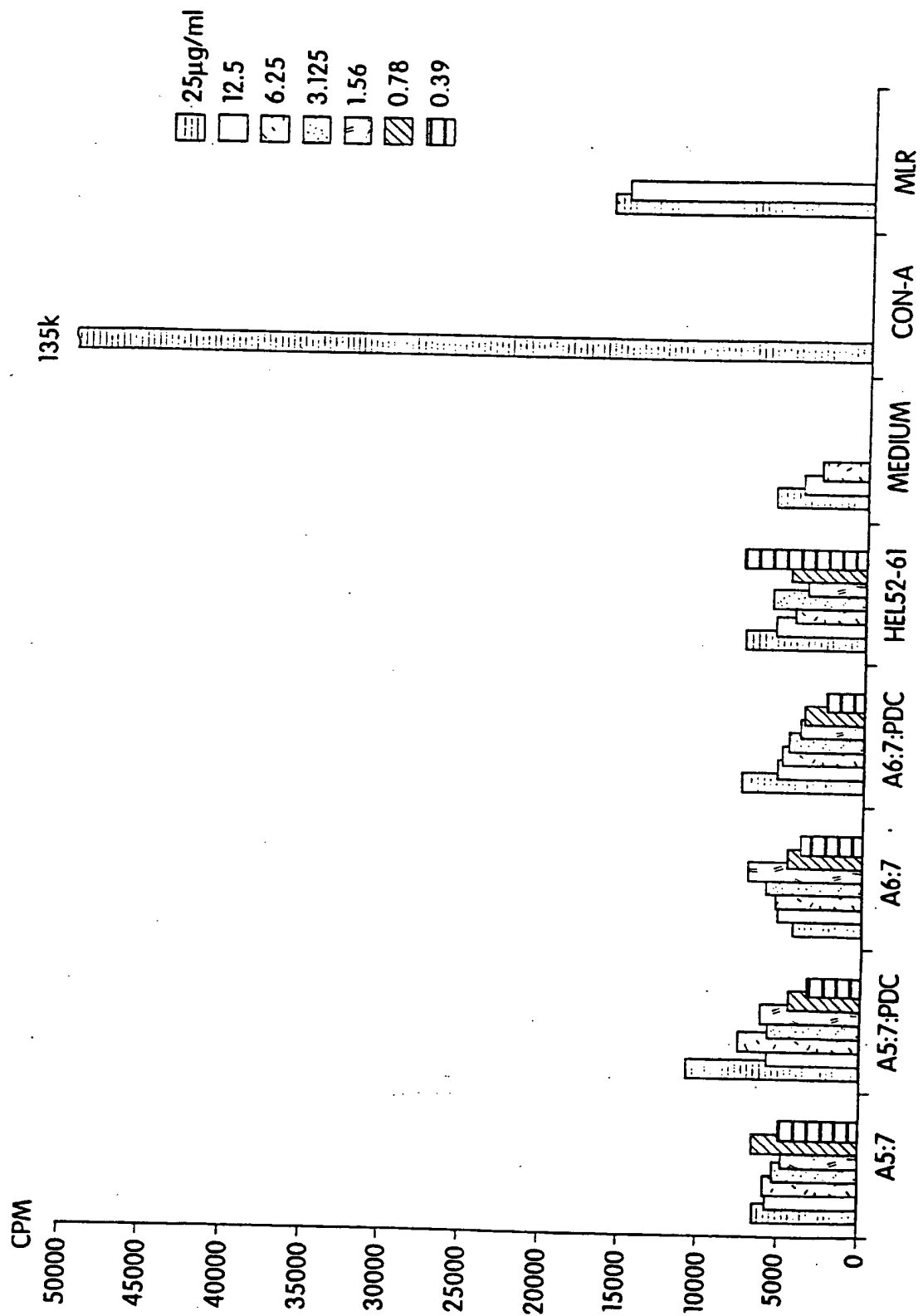


Fig. 6d

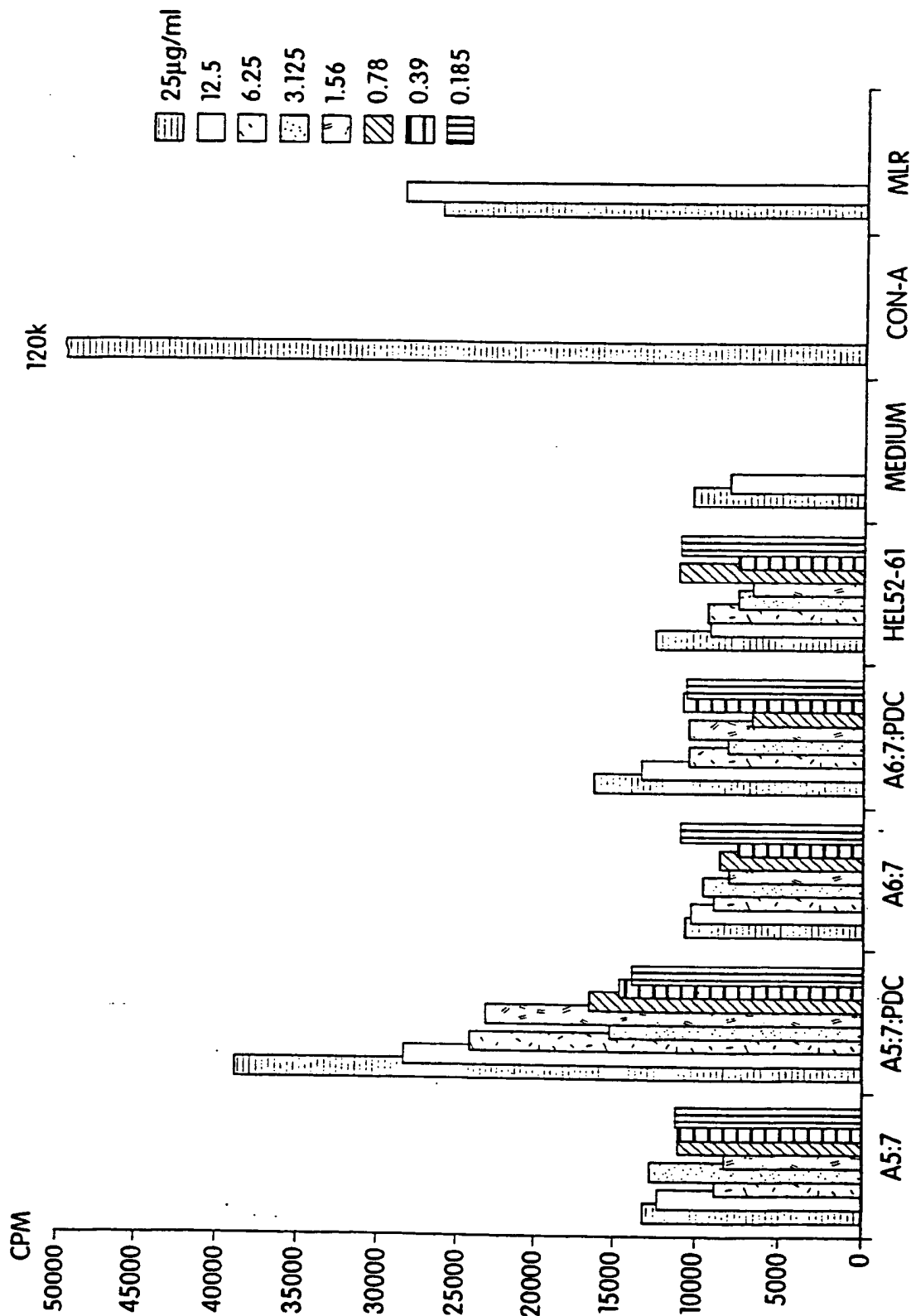


Fig. 6e



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US

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(57) Abstract

MHC-binding carrier peptides linked to catechol derivatives are provided which are recognized by urushiol-specific T lymphocytes. The compounds are useful in therapeutic compositions and methods for desensitizing individuals against contact sensitivity to haptens, such as urushiol of poison ivy/poison oak.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/04121

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C07K7/04 C07K14/435 A61K39/385

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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A	--- CHEM. RES. TOXICOL., vol. 6, no. 2, 1993, pages 216-222, NAZIH A. ET AL. 'Bihaptens with 5- and 6-Methyl-Substituted Alkylcatechols and Methylene Lactone Functional Groups: Tools for Hapten (Allergen or Tolerogen)-Protein Interaction Studies' see page 215 - page 216 --- -/-	

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Date of the actual completion of the international search

22 September 1995

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03. 11. 95

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A	WO,A,92 11859 (IMMULOGIC PHARMACEUTICAL CORP) 23 July 1992 see the whole document ---	
A	WO,A,92 07952 (IMMULOGIC PHARMACEUTICAL CORP ;MERCK & CO INC (US)) 14 May 1992 see the whole document -----	

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Information on patent family members

International Application No

PCT/US 95/04121

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